European Commission

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

Spodoptera exigua multicapsid nucleopolyhedrovirus (SeMNPV)

Active substance data

Volume 3 – Annex B.5 Analytical methods

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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INTRODUCTION

The company Andermatt Biocontrol GmbH submits the current dossier for the approval of the baculovirus (BV) *Spodoptera exigua* multi nucleopolyhedrovirus (SeMNPV) as a new microbial pest control agent (MPCA) and SPEXIT as its reference microbial pest control product (MPCP) to the European Authorities.

BVs used as MPCA in the EU are regulated as microorganism according to Regulation 1107/2009[1]. Data requirements for the registration of BVs as an active substance and their products are laid down in part B of the regulation documents 283/2013 [2]and 284/2013 [3]and the principles for evaluation and authorization of plant protection products contained microorganism according to regulation 546/2011[4].

BV isolates however, represent a unique case in which the wild type isolates are genetically heterogeneous (mixture of different genotypes or pool of isolates). These variations may influence in some biological properties, such as the virulence, but it has no consequences on the safety towards non-target organisms or the environment. Isolation of a single genotype is difficult and even not appropriated, since genetic variation is needed to account for variation in the target organisms and obtain better efficacy in the control of insect populations. Therefore, the BVs were not necessary evaluated at strain level (Sanco/0253/2008). [5]The high similarity between BVs justifies a general assessment at the level of the family *Baculoviridae*, considering species-specific information where necessary. The proposed procedure to include BVs at species level was adopted by the member states and the European Regulatory Authorities already in 2007, when the first BV species was included in Annex I, and for the REBECA proposal 2008[6], for a simplified inclusion of BVs on the species level into Annex I. Most of the formally required data are published and equal for all BVs, already assessed by MS and EU authorities and therefore, some data on the isolate or species level are not mandatory.

The BVs are included on species level in Annex I of directive 1107/2009 and the different pool of isolates were added after they have been evaluated to a separate list, to be maintained in the Review Report and to be amended by taking note in the Standing Committee (Sanco/0253/2008). This approach has been confirmed by a decision in the Standing Committee on May 15, 2007[7] were *S. exigua* NPV was listed at species level in Annex I. The experience that BVs present no risk for the environment have been confirmed by numerous studies during the last fifty years, since their first use as biocontrol agents. With regard to safety considerations, it is important to note that the whole *Baculoviridae* family are naturally present in our environment and are closely associated with their host occurrence. Therefore, their application in pest control would only produce a non-permanent fluctuation of the virus titre in the biotope of the pest insect. Due to their host specificity, BVs do not affect other organisms like vertebrates, arthropods other than their host species, microorganisms, or plants. BVs do not produce any metabolites at all.

For the BV specie *S. exigua* multicapsid nucleopolyhedrovirus (SeMNPV) a DAR with a reference isolate (Florida isolate SeNPV-F1, the first applied for) was approved in 2006 and the isolate SeNPV-F1 was listed on Annex I. Two new more isolates were further applied for at Member State level: the SeMNPV-SP2, approved in 2008 and the SeNPV-BV0004, approved in 2010. Conversely, the current dossier was based on the data already assessed by the MS and EU authorities:

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[1]*Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. Official Journal of the European Union L 309, 1-50.*

[2] Commission Regulation (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. Official Journal of the European Union L 93, 1-84.

[3]*Commission Regulation (EU) No 284/2013 of 1 March 2013 setting out the data requirements for plant protection products, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. Official Journal of the European Union L 93, 85-152.*

[4]*Commission Regulation (EU) No 546/2011 of 10 June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards uniform principles for evaluation and authorisation of plant protection products. Official Journal of the European Union L155, 127-175.*

[5]*SANCO/0253/2008 rev. 2, 22 January 2008. Guidance Document on the assessment of new isolates of baculovirus species already included in Annex I of Council Directive 91/414/EEC.*

[6]*Ehlers RU., 2011 Regulation of Biological Control Agents and the EU Policy Support Action REBECA. In: Ehlers RU. (eds) Regulation of Biological Control Agents. Springer, Dordrecht.*

[7]*Review report for the active substance Spodoptera exigua nuclear polyhedrosis virus. Finalised in the Standing Committee on the Food Chain and Animal Health at its meeting on 15 May 2007 in view of the inclusion of Spodoptera exigua nuclear polyhedrosis virus in Annex I of Directive 91/414/EEC. Spodoptera exigua NPV SANCO/T14/2007 - rev. final1 12 March 2007.*

- The previous DAR document for the approval of a new active substance SeNPV-F1 submitted by Mitsui Agri Science International S.A and evaluated by The Netherlands in 2007.
- The evaluation report of the new isolate of SeMNPV, BV0004 previously submitted by the company Andermatt Biocontrol GmbH and evaluated by the Netherlans in 2010.

Active substances are approved for maximum period of 10 years under Directive 91/414/EEC[8]. The active substance SeMNPV was under programme of renewal Regulation EU 686/2012 (AIR-III programme[9]). According to draft working document AIR III renewal programme SANCO/2012/11284[10], *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[11] 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.

In this RAR, the information submitted regarding *Spodoptera exigua* multicapside nucleopopyhedrovirus (SeMNPV) is evaluated as new active substance, therefore, all information is considered and evaluated as new.

Literature reference included by the applicant comes from a literature search according to EFSA (2011) [12]in order to identify relevant recent published peer reviewed references covering the last 10 years. The RMS has also included relevant studies considered important to support the application for the approval of *Spodoptera exigua* multipolyhedrivirus (SeMNPV) genotype pool BV-0004 and the microbial product SPEXIT.

For clarity, the following definitions are used in this RAR:

Infection: The introduction or entry of a pathogenic microorganism into a susceptible host, whether or not it causes pathological effects or disease. The organism must enter the body of the host, usually the cells, and be able to reproduce to form new infective units. Simply ingesting a pathogen does not imply infection.

Infectivity: The characteristics of a microorganism that allow it to infect a susceptible host.

Pathogenicity: the capacity of the virus to enter the host, establish infection, reproduce, and cause death (measured in terms of dose–mortality metrics).

Virulence: the time elapsing between initial infection and after the death of the host.

[8] *Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market. OJ L 230 of 19.8.1991.C.*

[9] *Programme of renewal Regulation EU 686/2012 (AIR-III programme).*

[10] *SANCO/2012/11284 –rev. 22, December 2018. Draft working document AIR III renewal programme.*

[11] *Commission implementing regulation (EU) No 844/2012, of 18 September 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 of the European Parliament and of the Council concerning the placing of plant protection products on the market.*

[12] *Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009. EFSA Journal 2011;9(2):2092.*

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

B.5.1.1 Methods for the identification of the microorganism

The occlusion bodies of SeMNPV are counted under the light microscope at 100-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), according to OECD 2002. 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.

1) Quantification method: Occlusion body counts. The most prominent characteristic is the formation of occlusion bodies (OB). In the past, the family was divided into Nucleopolyhedroviruses (NPV) and Granulovirus (GV) and the classification was based on the morphology of the occlusion body (OB). The OBs are crystalline matrices embedding the virion(s) and serve to protect the virions against damaging environmental conditions and allow virions to remain viable for many years **(Figure MA B.5.1.1-1A).** The occlusion bodies of NPV contain many enveloped virion and are polyhedra-like. Nucleopolyhedroviruses are 40-140 nm in width and 250-400 nm in length **(Figure MA B.5.1.1-1B)**. The NPV matrix protein polyhedrin is genetically and serologically closely related to the granulin, the matrix protein of GV (OECD, 2002).

Scanning electron microscopic observation of the SeMNPV polyhedron showed that the virus polyhedra were polygonal, nearly spherical and irregular, and the surface was smooth (**Figure MA B.5.1.1-1A).** The morphology of SeMNPV can be studied under the electron microscope in ultrafine sections **(Figure MA B.5.1.1-1B**).

The virus titre in a purified NPV suspension can be 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 (100-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, figure MA B.5.1.1-2**) with a depth of 0.02 mm.

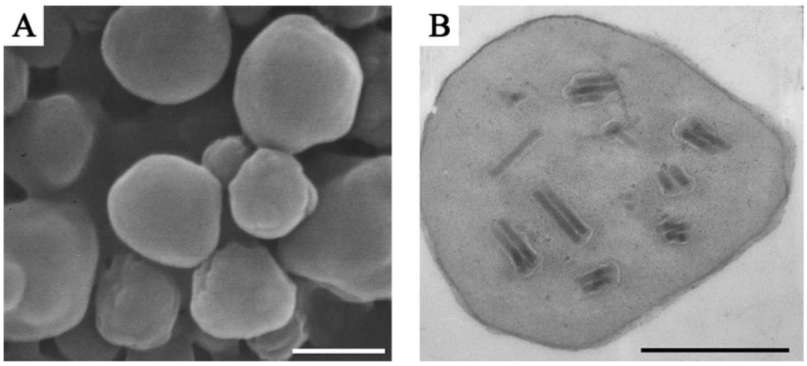

Figure MA B4.1.1-1. Micrographs of SeMNPV-QD (A) Scanning electron-micrographs of occlusion bodies (OBs); Bar=1.0μm; (b) Transmission electron-micrographs of OBs; Bar=500nm., (Chen et al., 2019).

The OB isolation protocols are generally based on several consecutive treatments of death insects with SDS and NaCl treatments with centrifugation. BVs pellet at the end of OBs isolation. After this, polyhedra can be quantified with an improved hemocytometer (Hausser Scientific, improved Neubauer hemocytometer, 0.100 mm deep) under a light microscope for detection the polyhedra suspension (**Figure MA B.5.1.1-2**).

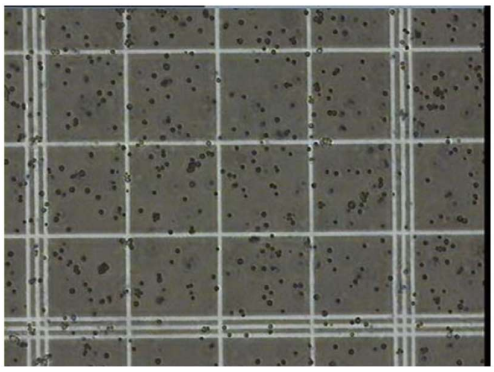

Figure MA B.5.1.1-2 The polyhedral under light microscopy on a Neubauer hemocytometer (Toprak, 2004).

2) Genotypic characterization (Molecular identification)

Zamora-Avilés et al., 2017 study describes the methodology for the genotypic and phenotypic characterization and differentiation of SeMNPV isolate Bv0004 from SPEXIT ppp.

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 four Mexican isolates (SeSINS, SeSLP6, SeSLPS and SeSIN6) and the three commercial isolates (SeSP2, SeUS2 and SeUS1) contained in Vir-X (SeSP2), Spod-X (SeUS2) and **SPEXIT (SeUS1)** with the restriction endonuclease BglII as follows:

Restriction endonuclease analysis of viral DNA. Viral DNA from a pool of larvae was obtained by dissolving 10^9 OBs with the addition of 100 ml of 0.5 M Na2CO3 and 50 ml of 10% (wt/v) SDS and incubating at room temperature for 3 min. Undissolved OBs and other particles were pelleted by centrifugation at low speed (6,000 g) for 5 min. The supernatant containing the virions was incubated with proteinase K at 50 ºC for 2.5 h. Viral genomic DNA was extracted with phenol and chloroform, precipitated by the addition of 0.1 volume 3 M sodium acetate (pH 5.2) and two volumes of 96% ethanol, and finally washed with 70% ethanol and suspended in 30 ml water. For REN analysis, genomic DNA was mixed with 10U of BglII (Promega, Madison, WI) and incubated for 4–8 h at 37 ºC. DNA reactions were electrophoresed in 0.7% TAE (Tris, acetate, and EDTA) agarose gels with 0.12 mg/ml ethidium bromide at low voltage (20 V) for 14 h.

Digestion with BglII resulted in a characteristic DNA profile for the Mexican isolates (SeSIN6, SeSIN8, SeSLP6, and SeSLP8), as indicated by the presence of the marker fragment (**Figure MA B.5.1.1-2**, 25 kb; **Figure MA B.5.1.1-2**.), absent in the corresponding profile of the reference isolates SeSP2 and SeUS2. Moreover, SeSP2-BglII isolate showed two BglII fragments of 15 and 2.4 kb (**Figure MA B.5.1.1-2A**; letters b and c, respectively) that were absent in the Mexican isolates, whereas SeUS2-BglII presented a marker fragment of 9.5 kb that was absent in the Mexican isolates. However, when compared with the SeUS1 isolate (**Figure MA B.5.1.1-2**.), the Mexican isolate BglII profiles presented a more similar pattern of bands, and a number of submolar bands (**Figure MA B.5.1.1-2, B).** Overall, REN profiles contained a number of submolar bands (**Figure MA B.5.1.1-2A–C),** indicating that the isolates comprised genotypic variants in different proportions. Restriction endonuclease analysis profiles of SeUS1, SeUS2, and SeSP2 isolates 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 (Muñoz et al. 1998).

SeMNPV isolate SeUS1 can be differentiated by restriction endonuclease analysis from two commercial SeMNPV isolates and other four Mexican isolates.

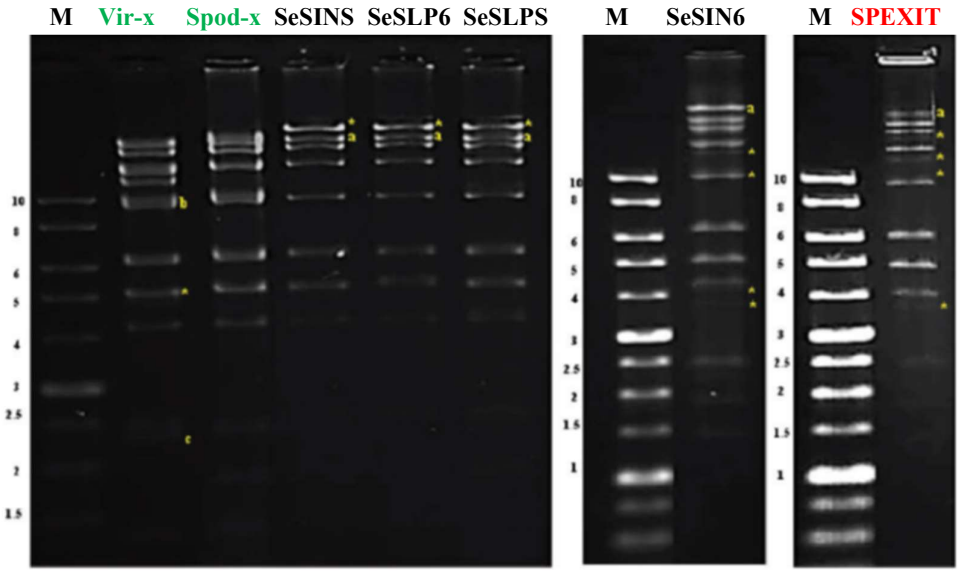

Figure MA B.5.1.1-2. Restriction endonuclease analysis profiles with BglII of DNA from seven isolates (SeSP2 (VIR-EX product), SeUS2 (Spod-x product), SeSIN8, SeSLP6, SeSLP8, SeSIN6 and SeUS1 (SPEXIT product). Lane M is the molecular marker size 1Kb. Yellow lettered fragments are RFLP diagnostic fragments for the genotypes (d-f). Asterisks in yellow represent submolar fragments visible in the profiles. **(Zamora-Avilés 2017).**

3) 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.
In this document, RMS refers to **pathogenicity** as the capacity of the virus to enter the host, establish infection, reproduce, and cause death (measured in terms of dose–mortality metrics), whereas **virulence** is taken to mean the time elapsing between initial infection and after the death of the host.

Determination of Dose–Mortality and Dose–Time Characteristics

- 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. For this, newly molted second instars is starved for 16–20 h and allowed to drink from an aqueous suspension containing OBs, 10% sucrose, and 0.01% (wt/v) Fluorella Blue. Seven viral doses containing 0.3, 1, 3, 9, 27, 81, and 243 OBs per larva of each viral inoculum is prepared to produce mortalities between 5% and 95%. Five to seven of these doses are selected for statistical analysis, as described by Murillo et al. (2007). The different suspensions and an OB-free solution is supplied to three batches of larvae, each comprising 24 individuals, for each virus treatment. Larvae that ingested droplets within 10 min were individually transferred to 24- well tissue culture plates containing diet and incubated at 25±2ºC. Mortality is recorded every 24 h for 7 days.
- **Pathogenicity**, as **LD_{50}** based on an average ingested volume of 0.33 ml per larva in this instar (Chaufaux and Ferron 1986).
- **Time–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 (Murillo et al. 2006a). Batches of 24 individual larvae are incubated following the droplet-feeding method described above and individually transferred to 24-well tissue culture plates containing diet and reared at 25±2ºC. The mortality was recorded every 8 h for 7 d. Bioassays are performed independently three times.

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 (Murillo et al. 2003). 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 Neuberger chamber.

B.5.1.2 Methods for providing information on possible variability of seed stock/active microorganism

RMS comments: 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 (Zamora-Avilés 2017).
- 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 (Zamora-Avilés 2017).

B.5.1.3 Methods to differentiate a mutant of the microorganism from the parent wild strain

RMS comments. 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.

B.5.1.4 Methods for the establishment of purity of seed stock from which batches are produced and methods to control that purity

RMS comments: 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 (Zamora-Avilés 2017).
- 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 (Zamora-Avilés 2017).

B.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

Quantification method for OBs counts (MA B.4.1.1).

RMS comments: 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 in MP Vol.3 B.5 for SPEXIT.

B.5.1.6 Methods for the determination of relevant impurities in the manufactured material

RMS comments: There is no provided information. On the basis of 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.

B.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

RMS comments: 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 for MPCP, see MP Vol.3 B.5 for SPEXIT.

B.5.1.8 Methods to determine storage stability, shelf-life of the microorganism, if appropriate

RMS comments: There is no provided information.
BVs are considered stable during storage under appropriate conditions where they remain inactive. Genetic changes are linked to a loss in infectivity, which results in reduced fitness followed by extinction in the field.
Defective interfering (DI) viruses are commonly produced by many viruses as a consequence of passage in cell culture, especially at high multiplicity. They consist of populations that have varying amounts of their genomes deleted including at least one essential gene thereby rendering them defective. However, they retain the signals that are required for genome replication and packaging. The DI populations often predominate and can result in severe declines in viral titers. It is thought that they out compete (interfere with) the wt virus replication because their genomes are smaller and thus are more efficiently replicated and consequently can greatly outnumber the helper virus.
This resulted in not only a reduction in the original phenotype, but also led to a delay in DI particle formation. It was suggested that a process that involves the insertion of transposons followed by deletion of sequences between the inserted transposons forms DIs and this may be an important step in DI formation.

The stability of the microorganism need to be evaluated phenotypic and genotypically according to provided method (B4.1.1)
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. Therefore, no information is required for the MPCA. Methods for MPCP, see MP Vol.3 B.5 for SPEXIT.

B.5.2 METHODS TO DETERMINE AND QUANTIFY RESIDUES (VIABLE OR NON-VIABLE) OF THE ACTIVE MICROORGANISM

The nature of the product and its active substance are not adequately described and assessed by applying the term ‘residue’, since this definition commonly implies a toxicological or environmental concern of the residual deposit of a plant protection product, which is not attributable to SeMNPV, for following reasons:

- SeMNPV is of natural origin and not genetically modified. It belongs to the family of baculoviruses which are naturally present in our environment. Occurence of SeMNPV is closely linked to the occurence of its host, *Spodoptera exigua*. Therefore, their application in pest control means only a fluctuation of the virus titre in the biotope of the pest insect. BVs are highly arthropod-specific viruses which are not harmful to non-arthropods, including domestic animals and man.
- The experience that contact of BVs with man or animals does not involve any risk for their health has been confirmed by numerous acute and subacute toxicity studies. In addition, test on mammalian cell cultures as well as on mutagenicity, teratogenicity and carcinogenicity all gave negative results (refer to Annex II, Section 3).
- BVs do not produce toxins or secondary metabolites of toxicological concern.
- BVs are unable to enter plant tissues and to infest them. They are also unable to multiply on plant surfaces.
- BVs are rapidly inactivated by the UV-portion in sunlight. Stable virus deposits on plant surfaces, therefore, are not assumed.
- In general, the period between application of the formulation and harvest is several days and certainly enough for avoiding relevant residues on the harvested crop parts.
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In summary, 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.

Quantification of infective SeMNPV follows a method originally described for detection of *Helicoverpa armigera* NPV in soil (Richards and Christian, 1999). The quantity of virus in soil is determined by incorporating known amounts of soil into artificial insect diet. The described procedure can be adapted to a bioassay using *S. exigua* larvae (Kessler 2006b, submitted in Doc J MA 1.4.1) instead of *H. armigera* larvae. Results from soil samples are quantified using a standard with sterilized soil and known amounts of virus suspension. Instead of soil samples, the same procedure should work to determine the amount of infective SeMNPV from leaves, fruit surfaces, or water. To this end, leaves and fruit surfaces are washed in a defined amount of water or buffer solution. The resulting washing suspension is then used in the bioassay as described above.

Alternatively, de Moraes et al. (1999) presented a method to isolate *Anticarsia gemmatalis* NPV from soil using magnetic-capture hybridization. An adaptation of this method for SeMNPV using a SeMNPV-specific probe should be possible.
A method to quantify BVs in water was developed by Jehle (2004). Purification of the OBs can be performed as described. For identification, the PCR method has to be adapted for SeMNPV. For virus quantification, adapted quantitative Real-Time PCR (Jehle, 2004) or a bioassay as described by Kessler (2006b, submitted in Doc J MA 1.4.1) can be used.

Summarizing, different methods are described to determine SeMNPV. 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.

B.5.2.1 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)
RMS comments: There is no provided information.
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.

B.5.2.2 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)
RMS comments: There is no provided information.
SeMNPV is not known to produce any human pathogens, no analytical methods are required.

B.5.2.3 Methods to identify any contaminating microorganisms of the preparation
See confidential Annex C.

B.5.2.4 Methods used to determine the storage stability and shelf life of the preparation
See confidential Annex C.

B.5.3 METHODS TO DETERMINE AND QUANTIFY RESIDUES (VIABLE OR NON-VIABLE) OF THE MICROORGANISM AS MANUFACTURED AND FOR THE ANALYSIS OF THE PREPARATION

B.5.3.1 Methods to determine and quantify residues (viable or non-viable) of the microorganism

The nature of the product and its active substance are not adequately described and assessed by applying the term 'residue', since this definition commonly implies a toxicological or environmental concern of the residual deposit of a plant protection product, which is not attributable to SeMNPV, for reasonspointed above.

B.5.3.1.1 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

Please refer to B.5.3.1.

B.5.3.1.2 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

SeMNPV does not produce any metabolites.

B.5.3.2.2 Methods to determine and quantify residues (viable or non-viable) of the microorganism for the analysis of the preparation

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.

B.5.3.2.2.1 Food and feed (where relevant)

Please refer to B.5.3.2.2.

B.5.3.2.2.2 Animal tissue (where relevant)

Please refer to B.5.3.2.2.

B.5.3.2.2.3 Water (where relevant)

Please refer to B.5.3.2.2.

B.5.3.2.2.4 Air (where relevant)

Please refer to B.5.3.2.2.

B.5.3.2.2.5 Analytical methods for amount or activity of proteinaceous products (where relevant)

SeMNPV does not produce any proteinaceous product.

B.5.4 IDENTIFICATION OF NECESSARY FURTHER INFORMATION

No further information is required.

B.5.5 REFERENCES RELIED ON

A literature search according to EFSA guidance (2011) was conducted in January 2018 covering the last 10 years. The literature research was carried out using the search-engine ProQuest DialogTM. After rapid assessment based on title and abstract; no reference was submitted to a detailed assessment of full text documents. No references were considered relevant relating residues. For more details please refer to Jakubowska (2018, provided in KMA 6/01).

Cited references

Report KMA 6/01 – Jakubowska (2018)

Literature review on *S. exigua* multiple nucleopolyhedrovirus (SeMNPV): Residues in or on treated products, food and feed

Not published

Summary: Not applicable

RMS comments:

- RMS has considered all document as new information on the current Draft Assessment Report for the new microbial pest control agent SeMNPV.
- In the opinion of the RMS, the literature research made by the applicant according to EFSA 2011 guidance covered the most relevant news for SeMNPV. The RMS has also included some new references considered important for the evaluation.

Data point	**Author(s)**	**Year**	**Title** **Owner, Report No.** **Source (where different from owner)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Data protection claimed Y/N**	**Justification if data protection is claimed**	**Owner**
MA B.4.1.1/01	OECD	2002	Consensus document on information used in the assessment of environmental applications involving baculoviruses not available, not applicable OECD Organisation for Economic Co-operation and Development, 2002 Not available; Not applicable. GLP/GEP: no Published: yes	N	N	not protected	
MA B.4.1.1/02	Yingjian Chen, Benxiang Qia, Guiling Zheng, Yuan Zhang, Fei Deng, Fanghao Wan, Changyou Li	2019	Identification and genomic sequence analysis of a new Spodoptera exigua multiple nucleopolyhedrovirus, SeMNPV-QD, isolated from Qingdao, China. Journal of Invertebrate Pathology 160 (2019) 8–17.	N	N	not protected	-

Data point	Author(s)	Year	Title Owner, Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
MA B.4.1.1/03	Toprak, U., Ş. Bayram, and M.O. Gürkan,	2005	Gross pathology of SpliNPVs and alterations in Spodoptera littoralis Boisd. (Lepidoptera: Noctuidae) morphology due to baculoviral infection. J. Agric. Sci., 11 (1): 65-71.				
MA B.4.1.1/04 MA B.4.1.2/01	Zamora-Avilés N. Zamora-Avilés, R. Murillo, R. Lasa, S. Pineda, J. I. Figueroa, A. Bravo-Patiño, O. Díaz, J. L. Corrales, and A. M. Martínez	2017	Genetic and biological characterization of four nucleopolyhedrovirus isolates collected in Mexico for the control of Spodoptera exigua (Lepidoptera: Noctuidae),” J. Econ. Entomol., vol. 110, pp. 1465-1475				
MA B.4.1.1/05	Murillo, R., Mun˜ oz, D., Ruı´z-Portero, M. C., Alca´ zar, M. D., Belda, J. E., Williams, T. & Caballero, P.	2007	Abundance and genetic structure of nucleopolyhedrovirus populations in greenhouse substrate reservoirs. Biol Control 42, 216–225.	N	N	not protected	
MA B.4.1.1/06	Chaufaux, J., and P. Ferron.	1986	Sensibilite´ differente de deux populations de Spodoptera exigua Hub. (Lep., Noctuidae) aux baculovirus et aux pyre´throodes de synthe`se. Agronomie 6: 99–104.				
KMA 4.2/01 MA B.4.2/01	Richards, A.R., Christian, P.D.	1999	A rapid bioassay screen for quantifying nucleopolyhedroviruses (Baculoviridae) in the environment. not available, not applicable Journal of Virological Methods, 82, 63-75 GLP/GEP: no Published: yes	N	N	not protected	-

Data point	**Author(s)**	**Year**	**Title** **Owner, Report No.** **Source (where different from owner)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Data protection claimed Y/N**	**Justification if data protection is claimed**	**Owner**
KMA 4.2/02 MA B.4.2/02	De Moraes, R.R., Maruniak, J.E., Funderburk, J.E.	1999	Methods for Detection of Anticarsia gemmatalis Nucleopolyhedrovirus DNA in Soil not available, not applicable Applied and Environmental Microbiology, 65, 2307-2311 GLP/GEP: no Published: yes	N	N	not protected	-
KMA 4.2/03 MA B.4.2/03	Jehle, J.	2004	Validation of an analytical method for the determination of Cydia pomonella granulovirus in surface water Andermatt Biocontrol GmbH, CPGV/2004-01 DLR-Rheinpfalz, Neustadt, Germany GLP/GEP: no Published: no	N	N	not protected	ABA

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