# *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.1 Identity

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 Suisse AG (new Swiss subsidiary of Andermatt Biocontrol AG) 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-

[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. final 12 March 2007.*

Bv0004, approved in 2010. Conversely, the current dossier was based on the data already assessed by the MS and EU authorities:

- 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, Bv-0004 previously submitted by the company Andermatt Biocontrol GmbH and evaluated by the Netherlands' in 2010.

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

[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.*

## B1. IDENTITY OF THE MICROORGANISM

SeMNPV is a BV belong to the family *Baculoviridae*, which are arthropod-specific, enveloped viruses with a circular double-stranded DNA genome. BVs exclusively have been isolated from arthropods, primarily from the three insect orders *Lepidoptera*, *Hymenoptera*, and *Diptera* (OECD, 2002). 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 naturally occurring virus worldwide and acts highly specific against larvae of the beet armyworm, *Spodoptera exigua*, therefore, the presence of SeMNPV in the environment is linked to the presence of the host, *S. exigua*. Thus, its application in pest control means only a fluctuation of the virus titre in the biotope of the pest insect (Krieg, 1976). The SeMNPV strain Bv-0004 in use was originally isolated in China (Jianfeng, 2005). It is not supposed to have any harmful effects on organisms not belonging to the genus *Spodoptera* SeMNPV does not produce antibiotics and secondary metabolites of toxicological and/or environmental, or ecotoxicological concern. Neither SeMNPV active ingredient produce nor the end-use product (SPEXIT) contains chemical compounds of critical toxicological, environmental, or ecotoxicological concern.

## B.1 IDENTITY OF THE MICROORGANISM

### B.1.1 APPLICANT

| | |
|---|---|
| **Company:** | **Andermatt Biocontrol Suisse AG (new Swiss subsidiary of Andermatt Biocontrol AG)** |
| **Address** | Zellerstrasse 9<br>79618 Rheinfelden<br>Germany |
| **Contact point:** | [redacted] |

### B.1.2 PRODUCER

Confidential information, see Volume 4, Annex C

### B.1.3 NAME AND SPECIES DESCRIPTION, STRAIN CHARACTERISATION

#### B.1.3.1 Accession number in culture collection

The BV preparation is deposited since 2006 in the German Collection of Microorganisms and Cell Cultures (DSMZ), Mascheroder Weg 1b, D-38124 Braunschweig, Germany. The Reference number for the mixture of genotypes is BV-0004 (Schönfelder, 2006). The mix of genotypes derives from a wild type and is being used as such, without mutants or genetic modifications. The mixture of genotypes that is used as active ingredient in the product SPEXIT was characterised by analysis of the restriction fragment patterns after digestion with different restriction endonucleases (Jhele 2007).

The SeMNPV strain BV-0004 was isolated by Institute of Zoology, Chinese Academy of Science from several *S. exigua* larval cadavers infected with nuclear polyhedrosis disease in green capsicum field, Huainan City, Jiangsu province, China in 2000 (Jianfen, 2005).

**RMS comments:**

There is no available information on the culture deposit on document Schönfelder, 2006 (KMA 1.3/04). The accession number does not correspond with any available culture strain. According to Rule 9 of the Budapest Treaty the DSMZ keeps strict secrecy regarding the deposit, its nature and the depositor. Cultures deposited for patent purposes are handled by a separate department within the DSMZ, and the strain and the information concerning the strains, are handled confidentially and independently of the main collection. Nevertheless, strains deposited under the Budapest Treaty will, by no means, be included in the DSMZ catalogues or any publicly available lists. The statement "the strain is deposited since 2006 in the German Collection of Microorganisms and Cell Cultures (DSMZ)" seems to be insufficient. The applicant has only provided the DSMZ confirmation deposit document of the BV commercial preparation in Schöenfelder 2006 (Document KMA 1.3/04).

Further information regarding the isolate deposit on the international depositary authority, DSMZ are required:

RMS requires information on BV-0004 isolate deposit DSMZ:

i. Depositor.
ii. Origen of the isolate.
iii. Host isolation.
iv. Receipt and acceptance.

v. Identification of the microorganism.
vi. Scientific description and proposed taxonomic designation.
vii. Stability statement.

Isolate-specific information has to be provided for the active substance (according to Principles Uniform EU No 564/2011 part II, 2.2. Biological, physical, chemical, and technical properties-**Origen of the isolate).**

**B.1.3.2 Scientific name and taxonomic grouping, i.e. family, genus, species, strain, serotype, pathovar or any other denomination relevant to the microorganism.**

| | |
|---|---|
| **Indigenous or non-indigenous:** | SeMNPV is a natural enthomopathogenic nucleopolyhedrovirus, naturally present in the environment |
| **Wild type:** | Yes, the strain was isolated from armyworm larvae. |
| **Spontaneous or induced mutant:** | The strain is not described as a mutant |
| **Genetically modified according to Directive 2001/18/EC:** | No |

SeMNPV is a natural entomopathogen virus that belongs to the family *Baculoviridae* Group II Alphabaculovirus, (**Table MA B.1.3.2-01; figure MA B.1.3.2-02; figure MA B.1.3.2-03**) (Jehle *et al*., 2006, Rohrmann, 2013, Thèzè *et al*., 2014).

| | |
|---|---|
| *dsDNA virus* | |
| *Order* | *Unassigned* |
| *Family* | *Baculoviridae* |
| *Genera* | *Alfabaculovirus* |
| *Specie* | *Spodoptera exigua multiple nucleopolyhedrovirus (SeMNPV)* |
| *Isolate* | *SeMNPV BV-0004* |

**Table MA B.1.3.2-01** Taxonomic classification for SeMNPV according to ICTV 9th report (King *et al*., 2011).

During the biphasic replication cycle of BVs, two distinct viral phenotypes are formed: Occluded virions and budded virions differing in the origin and composition of their envelopes and their roles in the virus life cycle. ODVs are released from the inclusion bodies and infect midgut epithelium cells (first round of virus replication), then the newly produced nucleocapsid traverse the nuclear membrane, the cytosol and bud through the basal lamina of the midgut cells into the hemolymph. The now so called BVs acquire a new envelope and are responsible for the systemic cell-to-cell infection in host larvae (OECD, 2002).

The morphology of SeMNPV can be studied under the electron microscope in ultrafine sections (Yingjian Chen**,** 2019) **(Figure MA B.1.3.2-01**). The most prominent characteristic is the formation of occlusion bodies (OB). In the past, the family was divided into Nucleopolyhedrovirus (NPV) and Granulovirus (GV) and the classification was based on the morphology of the 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.1.3.2-01A).** The occlusion bodies of NPV contain many enveloped virion and are polyhedra-like **Figure MA B.1.3.2-01B)**. Nucleopolyhedroviruses are 40-140 nm in width and 250-400 nm in length. The NPV matrix protein polyhedrin is genetically and serologically closely related to the granulin, the matrix protein of GV (OECD, 2002).

The fact that BV species are named according to the OB morphology and the host leads to different problems: If two genetically different, but morphologically similar viruses are isolated from the same host, they will get the same species name irrespective of the genetic difference. On the other hand, the same virus genotype isolated from two different

hosts will get two different species names. Recently, a phylogenetic species criterion was proposed based on the similarity of three partial gene sequences, polh/gran, lef-8 and lef-9, (Jehle *et al*., 2006, Rohrmann, 2013). The lef-8 and lef-9 genes encode for subunits of the BV RNA polymerase, were identified in all completely sequenced BV, therefore, is suitable for studying BV phylogeny. The separation of lepidopteran-specific NPVs into groups I and II was made with the polh/gran gene, one of the most conserved genes in lepidopteran-specific BVs (**Figure MA B.1.3.2-02)** (Rohrmann, 2013).

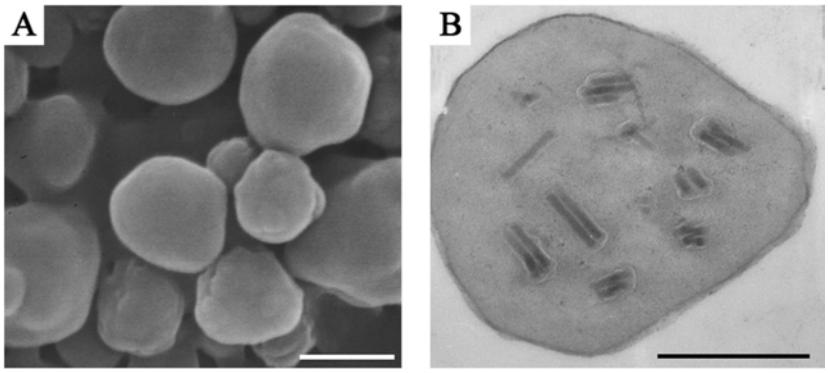


**Figure MA B.1.3.2-01.** Micrographs of SeMNPV **(A)** Scanning electron-micrographs of occlusion bodies (OBs); Bar=1.0 µm; **(B)** Transmission electron-micrographs of OBs; Bar=500 nm, (Yingjian Chen, 2019).

The OBs of Alphabaculovirus, Gammabaculovirus, and Deltabaculovirus show a polyhedral shape with a size of 0.5 to 5 µm, containing many virions. The OBs of Betabaculovirus show an ovicylindrical shape with a size of 0.3 × 0.5 µm, containing only one, rarely two or more virions. Baculoviridae genomes encode for 100 to 200 proteins, whereby thirty gene homologs form the BV core genes, which are shared among alpha-, beta-, gamma- and deltabaculoviruses. The conserved genes are involved any a variety of functions, including DNA replication, late gene transcription and virion structure. The matrix proteins (polyhedrin and granulin) of different *Baculoviridae* genera are serologically closely related.

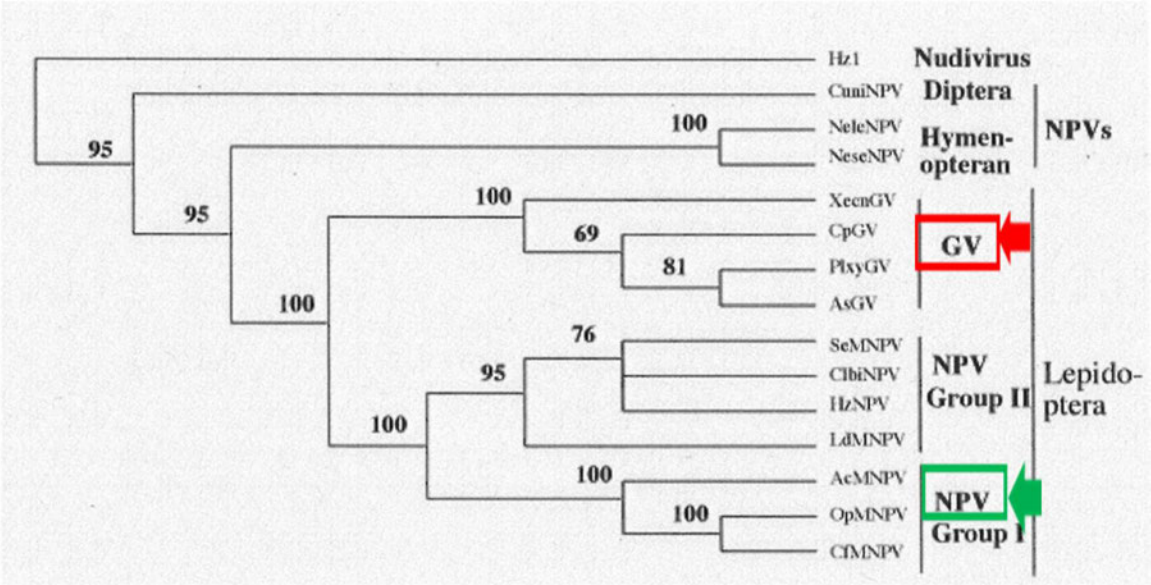


**Figure MA B.1.3.2-02.** Phylogenetic relatedness of LEF8 from selected BVs Neighbour joining; bootstrap analysis (1000 reps). Green arrow pointed to SeMNPV specie, and red arrow pointed the Bv *Cydia pomonella* granulovirus (CpGV) (Rohrmann, 2013).

Recent phylogenetic analysis (Yingjian Chen, 2019) based on 30 core genes have confirmed that the BVs family consists of four monophyletic groups. Based on phylogenetic, phenotypic and biological properties, the family

Baculoviridae can be divided into four genera **(Figure MA B.1.3.2-03)** which is also linked to the insect orders of the corresponding hosts and on the morphology: Alphabaculovirus (lepidopteran-specific nucleopolyhedroviruses (NPVs)), Betabaculovirus (lepidopteran-specific granuloviruses (GVs)), Gammabaculovirus (hymenopteran-specific ((NPVs)) and Deltabaculovirus (dipteran-specific (NPVs)) (Rohrmann, 2013). Among them, BVs within the genus Alphabaculovirus are divided into two groups, encompassing the **GROUP I** *Spodoptera exigua* multiple nucleopolyhedrovirus **(SeMNPV),** *S. frugiperda* multiple nucleopolyhedrovirus **(SfMNPV),** *S. litura* nucleopolyhedrovirus II **(SpliNPV-II**), *Agrotis segetum* nucleopolyhedrovirus (**AgseNPV**), and *A. ipsilon* multiple nucleopolyhedrovirus(**AgipNPV),** and **GROUP II** with *Autographa californica* multiple nucleopolyhedrovirus **(AcMNPV**)(**figure MA B.1.3.2-03**) (Rohrmann, 2013 et al., Yingjian Chen et al., 2019).

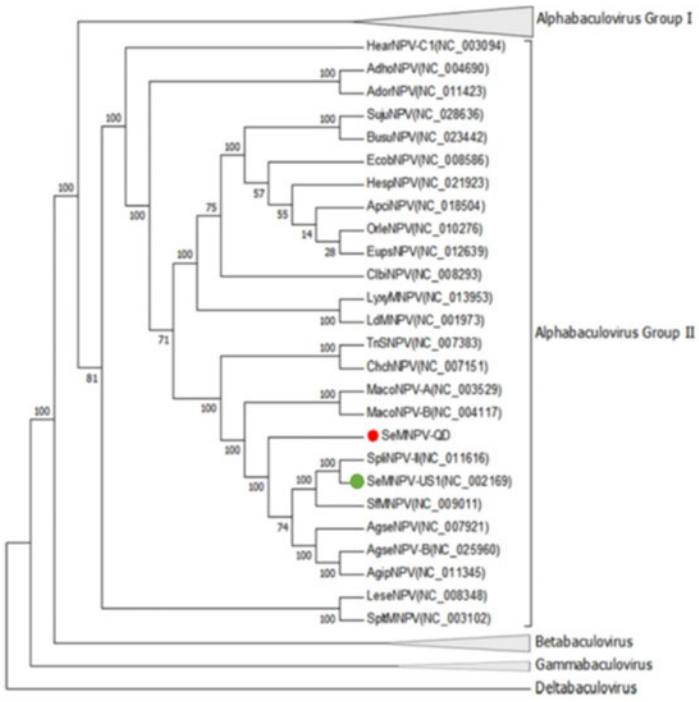


**Figure MA B.1.3.2-03.** Phylogenetic tree of SeMNPV-QD isolate (isolated from Qingdao, China, red dot) and Florida isolate (SeMNPV-US1, green dot) and other BVs. The maximum likelihood (ML) tree was generated based on the concatenated protein sequences of 38 core genes with fault parameters and percentage bootstrap values (1000 replicates) (Yingjian Chen, 2019).

**RMS comment:** According to the applicant, the isolate was originally isolated in China. More information and document confirmation is needed (place of isolated, year, host insect and/or crop…).

### B.1.3.3 Test procedures and criteria used for identification at strain level

BVs represents a unique case among microorganisms used in plant protection products (ppp), in that they consist of a mixture of different genotypes. The composition of this mixture depends among other factors on the genotype of the host used to multiply the BVs. Isolation of a single genotype is extremely difficult and even not desired since genetic variation is needed to account for variation in the target organisms. Therefore, the demand to evaluate microorganisms at strain level is not applicable[13]

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

Because of the BVs isolated from nature are generally a mixture of different genotypes variants, the morphological and genotypic discrimination at strain level are not possible. However, this variants can be cloned individually and their biological activities can be performed individually (Murillo *et al.* 2011). The genetic variability in the isolates should be detected and differences of the genotypes can be performed by randomly picking plasmid colonies after a PCR amplification. Restriction enzyme analysis of the isolate can be a starting point to detect its variability. Next, a cloning step to isolate genotypes, followed by their characterization at genetic and biological level. Variation in their replication speed and in the number of progeny virions produced can follow these. The effectiveness of SeMNPVs and the prevalence of disease in natural insect populations depends on the variation in OB pathogenicity and OB productivity, associated with each particular genotypes.

Morphological criteria are not suitable enough for the characterisation of the isolate, as all MNPVs have a very similar morphology. According to OECD 2002, the identification of BVs pool of isolates RFLP analysis is usually used. 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.

According to Heldens, 2007, the characterisation of the product SPEXIT was made by restriction endonuclease analysis (RFLP) of the viral DNA .The fingerprint profile obtained for SPEXIT product was compared with the fingerprint profile obtained for the isolate SeMNPV-US2, markers with the trade name Spod-X (Jehle and Matt-Schmid, 2007), **(Figure MA B.1.3.3-01)**. Both DNA profiles were also compared with known SeMNPV California isolate SeNPV-US1 (Heldens *et al*., 1996). California isolate was obtained from B.A. Federici Department of entomology, University of California at Riverside, USA in the form of polyhedral (Gelernter and Federici, 1986).

Restriction with *Bam*HI and *Xba*I did not reveal differences between the SPEXIT and Spod-X isolates. On the other hand, differences in the restriction patterns were observed after digestion with *Nde*I, *Pst*I, and *Bgl*II (**Figure MA B.1.3.3-01A)**. The comparison of these restriction patterns with published data revealed that the pattern of the SPEXIT isolate is identical to the type strain SeMNPV-US1 (**Figure MA B.1.3.3-01B)**, whereas the Spod-X pattern corresponds to SeMNPV-US2. On the bases of obtained fingerprint patterns can be concluded that the isolates used in the two commercial products SPEXIT and Spod-X can thus be distinguished by restriction mapping using the endonucleases *Nde*I, *Pst*I, and *Bgl*II.

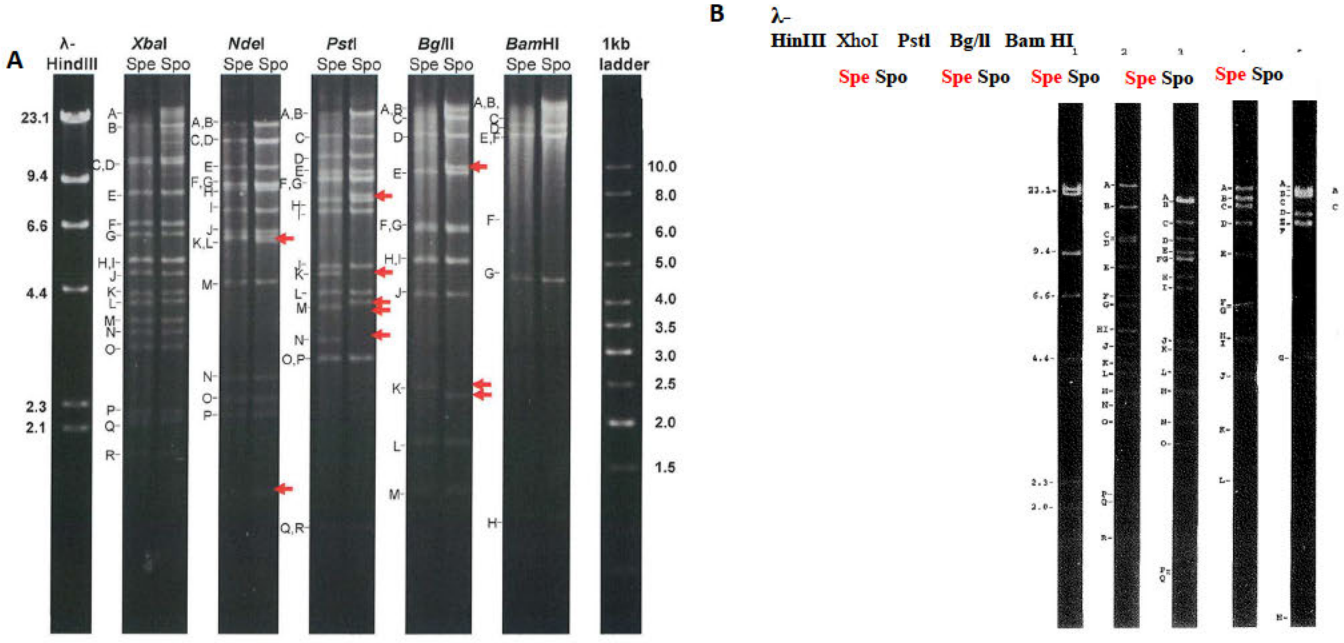


**Figure MA B.1.3.3-01. (A)** RFLP profiles of **SPEXIT** (lane Spe) and SeMNPV-US2 (**Spod-X**) (lane Spo) digested with Xbal, Ndel, Pstl, BglII, and BamHI in agarose gel 0.8%. **λ**-HinIII DNA and 1Kb ladder are markers. Restriction

fragments are lettered in order of size (Jehle and Matt-Schmid, 2007). **(B)** RFLP profiles of **SeMNPV-US1** digested with XoI, PstI, BgIII, and BamHI in agarose gel 0.8%. -HinIII DNA ladder is the markers (Caballero *et al*. 2001).

Different pool of isolates of SeMNPV have been isolated in different locations worldwide and have been compared biological and biochemically. Base on the RFLP analysis of the viral genomes, all of the SeMNPV wildtypes are closely related to SeNPV-US1 original from California **(Figure MA B.1.3.3-01B)**. This isolate have been propose as reference strain for SeMNPV (Caballero *et al*. 2001).

**Figure MA B.1.3-02** shows the RFLP genome analysis with BglII restriction enzyme for different SeMNPV isolates used as MPCA and compare the MPCP SPEXIT with VIR- EX, contained SeMNPV isolate SP2:

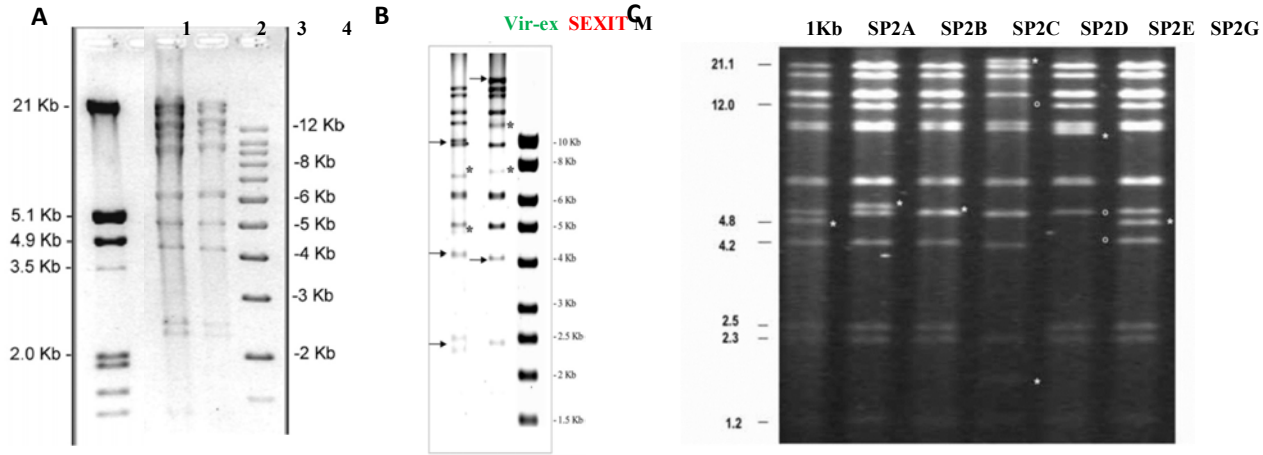


**Figure MA B.1.3.3-02. (A)** Agarose gel electrophoresis of BglII restriction analysis of the DNA samples (pooled samples from three repetitions Spanish isolates) after endonuclease restriction (lanes 3 and 4). Molecular size markers were lambda DNA/Ecoli RI+HinIII marker (lane1) and 1-Kb DNA ladder (lane4). (Lasa *et al*., 2008) **(B)** Agarose gel electrophoresis of BglII restriction analysis of the DNA samples from SeMNPV commercial products VIR-EX and SPEXIT. Polymorphic fragments are marketed with *. Lane M is -Kb DNA ladder (Elvira *et al*., 2013) **(C)** Agarose gel electrophoresis of DNA Spanish isolates (SP2A, SP2B, SP2C, SP2E, SP2F and SP2G) from SeMNPV OBs with enzyme BglII. Polymorphic fragments are marketed with * and band absence with. Left side has marker for -Kb DNA ladder (Zamora-Avilés *et al*., 2018).

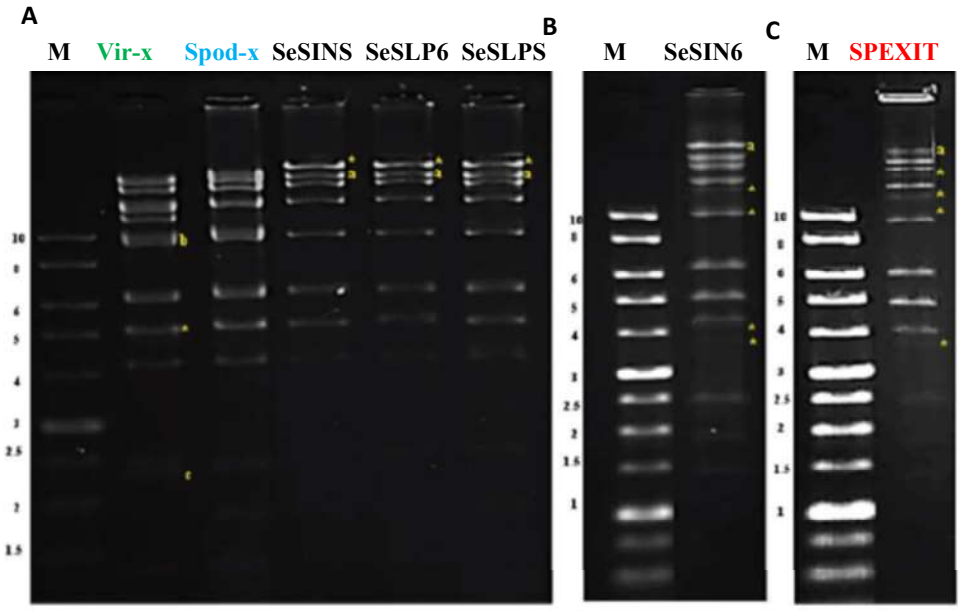

**Figure MA B.1.3.3-03.** Restriction endonuclease analysis profiles with BgIII of DNA from seven isolates (SeSP2 (**VIR-EX** product), SeUS2 (**Spod-x** product), SeSIN8, SeSLP6, SeSLP8, SeSIN6 and SeUS1 (**SPEXIT** product). Lane M is the molecular marker size 1KB. Yellow lettered fragments are RFLP diagnostic fragments for the genotypes (d-f). Asterisks in yellow represent sub molar fragments visible in the profiles. (Zamora-Avilés 2017).

The analysis of viral DNA was performed to confirm both identity and genomic integrity of the virus causing larval death after infections of seven instar larvae of *S. exigua* (four Mexican isolates (SeSINS, SeSLP6, SeSLPS and SeSIN6) and the three commercial isolates (SeSP2, SeUS1 and SeUS2) contained in Vire-x, Spod-x and SPAXIR with the restriction endonuclease BgIII. 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.1.3.3-03A**, 25 kb; **Figure MA B.1.3.3-03B**.), 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.1.3.3-03A**; 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.1.3.3-03C**.), the Mexican isolate BglII profiles presented a more similar pattern of bands, and a number of submolar bands (**Figure MA B.1.3.3-03A, B).** Overall, REN profiles contained a number of submolar bands (**Figure MA B.1.3.3-03 A–C),** indicating that the isolates comprised genotypic variants in different proportions.

**RMS comments:** The document (Jianfeng 2005, KMA1.3/05) is not considered valid for the description of a valid method for SeMNPV isolate Bv-0004 identification. This affirmation is based on the following points:

1) The restriction endonuclease map has not a proper DNA marker size: it doesn´t cover all the bands size of restriction analysis product.
2) There is no negative control with other related BV either with other SeMNPV virus.
3) The bands are not clear enough and the bands size is not possible to determine.
4) There is no blank lane.
5) There is no date of the experiment performer.
6) There is no information of the specific SeMNPV isolate used in the analysis.
7) The result analysis of the restriction endonuclease map was not provided.

- The RFLP obtained in Jianfeng 2005 cannot be compared to the RFLP obtained by Jehle in 2006 (KMA 1.3/07). There is no evidences of the identity of both BVs. On the other hand, according to Jehle 2007, the comparison of restriction patterns with published data revealed that the pattern of the SPEXIT isolate is identical to the type strain SeMNPV-US1 (**Figure MA B.1.3.3-01B).**
- 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 techniques 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.

**Viral structure identification**

Conventional purification and electron microscopy (EM) staining methods were traditionally applied to study the viral structures by standard. However, due to the fragility of BVs, the native virus structure was often significantly falsified (Wang *et al*. 2016). Among other viruses, (Wang *et al.*, 2016) surveyed the structure of SeMNPV using cryo-EM analysis providing novel insights into the near-native morphological structure of the BVs. An extended 'ovoid' shape instead of a 'rod' shape as described previously was found. Furthermore, imaging of intact BVs, revealed that the 'pocket' between the nucleocapsid and lateral envelope is most probably not empty, the pockets might be filled with

soluble content, and the envelope proteins, which appear as spikes perpendicular to the viral envelope, are present at both ends of the virus.

| | |
|---|---|
| **Reference:** | **Wang, Q., Bosch, B.-J., Vlak, J.M., van Oers, M.M., Rottier, P.J., van Lent, J.W.M. (2016)** Budded BVs particle structure revisited. |
| Report No.: | **KMA 1.3/10** |
| Guideline: | Published report. |
| GLP: | no |
| **Abstract:** | BVs are a group of enveloped, double-stranded DNA insect viruses with budded (BV) and occlusion-derived (ODV) virions produced during their infection cycle. BVs are commonly described as rod shaped particles with a high apical density of protein extensions (spikes) on the lipid envelope surface. However, due to the fragility of BVs the conventional purification and electron microscopy (EM) staining methods considerably distort the native viral structure. Here, we use cryo-EM analysis to reveal the near-native morphology of two intensively studied BVs, *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV) and *Spodoptera exigua* MNPV (SeMNPV), as models for BVs carrying GP64 and F as envelope fusion protein on the surface. The now well-preserved AcMNPV and SeMNPV BV particles have a remarkable elongated, ovoid shape leaving a large, lateral space between nucleocapsid (NC) and envelope. Consistent with previous findings the NC has a distinctive cap and base structure interacting tightly with the envelope. This tight interaction may explain the partial retaining of the envelope on both ends of the NC and the disappearance of the remainder of the BV envelope in the negative staining EM images. Cryo-EM also reveals that the viral envelope contains two layers with a total thickness of ≈ 6–7 nm, which is significantly thicker than a usual biological membrane (<4 nm) as measured by X-ray scanning. Most spikes are densely clustered at the two apical ends of the virion although some envelope proteins are also found more sparsely on the lateral regions. The spikes on the surface of AcMNPV BVs appear distinctly different from those of SeMNPV. Based on our observations we propose a new near-native structural model of BVs. |

**RMS comment:** The article is considered relevant as it provides information about the morphological specific properties of the species, using crio-EM of the vitrified BV suspension. The studio has revisited the morphology of virions SeMNPV, providing new insights on the ultrastructural organization and assembly of the BV virions.
The relevance and reliability of the findings in the paper are clear, those are considered as supplementary information for the current evaluation.

**Viral molecular identification**

RFLP or REN analysis were the standards for the comparison of different BV isolates. Newer research started to study genome data. The genetic variations may be linked to possible phenotypic effects in different isolates. Thézé *et al.* (2014) applied a comparative genomic analysis of among seven European SeMNPV isolates, differing in virulence, and found a similar genome size and content, but a high number of polymorphic sites, however with relatively few sites in the genomes that were potentially involved in functional changes. Based on Thézés *et al.* (2014) observations it was concluded, that BV populations naturally have high genomic variation at different levels of the interaction between virus and host during the course of an infection, and further suggested, that the differences in BVs virulence and transmission phenotypes involve multiple molecular pathways.

| | |
|---|---|
| **Reference:** | **Thézé, J., Cabodevilla, O., Palma, L., Williams, T., Caballero, P., Herniou, E.A. (2014)** Genomic diversity in European *Spodoptera exigua* multiple nucleopolyhedrovirus isolates. |
| Report No.: | **KMA 1.3/11** |
| Guideline: | Published report |
| GLP: | no |
| **Abstract:** | Key virus traits such as virulence and transmission strategies rely on genetic variation that results in functional changes in the interactions between hosts and viruses. Here, comparative genomic analyses of seven isolates of *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) with differing |

phenotypes were employed to pinpoint candidate genes that may be involved in host–virus interactions. These isolates obtained after vertical or horizontal transmission of infection in insects differed in virulence. Apart from one genome containing a *piggyBac* transposon, all European SeMNPV isolates had a similar genome size and content. Complete genome analyses of single nucleotide polymorphisms and insertions/deletions identified mutations in 48 ORFs that could result in functional changes. Among these, 13 ORFs could be correlated with particular phenotypic characteristics of SeMNPV isolates. Mutations were found in all gene functional classes and most of the changes we highlighted could potentially be associated with differences in transmission. The regulation of DNA replication (helicase, lef-7) and transcription (lef-9, p47) might be important for the establishment of sub lethal infection prior to and following vertical transmission. Virus–host cell interactions also appear instrumental in the modulation of viral transmission as significant mutations were detected in virion proteins involved in primary (AC150) or secondary infections (ME35) and in apoptosis inhibition (IAP2, AC134). BV populations naturally harbour high genomic variation located in genes involved at different levels of the complex interactions between virus and host during the course of an infection. The comparative analyses performed here suggest that the differences in BV virulence and transmission phenotypes involve multiple molecular pathways.

**RMS comment:** This scientific paper is considered relevant for the current evaluation as it provides information about the genetic diversity of the European strains belong to SeMNPV specie.
The genomic comparative analysis made on different SeMNPV viruses had provided valuable information on differences in virulence and transmission strategies among isolates. The article found the high genomic variation in located gens involved in interaction between virus and host during the infection process. This information will help to differentiate the specific strain Bv-0004 from other related European MPCA. It also provided for new specific technology for SeMNPV DNA sequencing, Genome assembly and annotation.

No details on the identification or the strain code of the virus were revealed, therefore, relationship of the eight evaluated strains to the SeMNPV strain Bv0004 was unknown. The data will be considered as supplementary information.

**RMS final comment on strain identity:** The target isolate would be convenient to identify and discriminate against other isolate with similar phenotypic and genotypic characteristics. The above study have pointed the main different between SeMNPV strains is the virulence, a key factor for PPP successful viruses, that need to have high pathogenicity and infectivity on the target host.
The different strains of SeMNPV isolates worldwide can be distinguished unequivocally from each other by one or more DNA restriction enzyme fragments. For example, for SeMNPV strains from three geographically very separate regions (from the United States, Spain, and Thailand), the PstI M fragment from each isolate served as a restriction fragment length polymorphic marker for their identification. Additionally, the biological activities of some of the various SeMNPV strains differed significantly from each other.

- 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 techniques 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.

### B.1.3.4 Common name or alternative and superseded names and code names used during the development

BVs are rod-shaped and enveloped and contain a circular double-stranded DNA genome (OECD, 2002). The valid name of the BV that is subject of this dossier is *Spodoptera exigua* multiple nucleopolyhedrovirus or multicapsid nucleopolyhedrovirus (SeMNPV) **(Table MA B.1.3.2-01).**

In the literature, the term *Spodoptera exigua* nucleopolyhedrovirus (SeNPV) is also used and refers to the same species.

Other BVs isolated from *Spodoptera 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 (Jehle *et al*., 2006) **(Figure MA B.1.3-02).**

#### B.1.3.5 Relationship to known pathogens

BVs are arthropod-specific viruses, with the majority of the host species belonging to the insect orders *Lepidoptera*, *Diptera* and *Hymenoptera*. SeNPV is only infective on few species within the family *Noctuidae*, most, if not all belonging to the genus *Spodoptera*. For details of the host range, please refer to Volume 3, Section 2, Point B.2.1.3.

SeMNPV as well as all other known BVs, i.e., CpGV-SC and SpliNPV, have been exclusively isolated from arthropods and not from other animals, humans or plants. They are not related to any known plant or human pathogen. Replication in animals other than arthropods or in plants was never observed for BVs. No adverse effects on human health has been observed indicating that the use of BVs is safe and does not cause any health hazards (OECD, 2002).

## B.1.4 SPECIFICATION OF THE MATERIAL USED FOR MANUFACTURING OF FORMULATED PRODUCTS

Confidential information, see Volume 4, Annex C.

### B.1.4.1 Content of the microorganism

Confidential information, see Volume 4, Annex C.

### B.1.4.2 Identity and content of impurities, additives, contaminating microorganisms

Impurities: Confidential information, see Volume 4, Annex C.

### B.1.4.3 Analytical profile of batches

Confidential information, see Volume 4, Annex C.

## B.1.5 REFERENCES RELIED ON

A literature search according to EFSA (2011)[14] was conducted to identify relevant recent published peer reviewed references covering the last 10 years (Gueli Alletti, 2018). The literature research was conducted on the search-engine ProQuest Dialog™. The data requirement "Biological properties of the micro-organism" was covered using a focused search encompassing BVs in general but focused on specific search terms related to biological properties. This focused search retrieved a large number of references (240) which were sorted manually for relevance for the data requirements. After a first check for relevance, 22 references were submitted to full text analysis. According to the full text analysis 17 references were regarded relevant for M-MA Section 1, Section 2 and Section 3 of this dossier. For all details on the selection process, please refer to the literature review report submitted in KMA 1.3/01.

From peer reviewed open literature, no essential new findings on the SeMNPV description or strain characterisation were identified by the applicant. Instead, a reference describing the SeMNPV structure in more details was found.

**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.

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| **Data point** | **Author(s)** | **Year** | **Title**<br>**Company Report No**<br>**Source (where different from company)**<br>**GLP or GEP status**<br>**Published or not** | **Vertebrate study**<br><br>**Y/N** | **Data Protection Claimed**<br><br>**Y/N** | **Owner** |
|---|---|---|---|---|---|---|
| KMA 1.3/06<br>MA B.1/01 | Krieg, A. | 1976 | Granulosis and nuclear polyhedrosis viruses: safety aspects concerning their production and application<br>Z Angew Entomol, 82, 129-134<br>Not available; Not applicable.<br>GLP/GEP: no<br>Published: yes | N | N | Public |
| KMA1.3/01<br>MA B.1/02<br>MA B.1.3.2/05<br>MA B.1.3.3/01<br>MA B1.3.2/05 | OECD | 2002 | Consensus document on information used in the assessment of environmental applications involving baculoviruses<br>not available, not applicable<br>OECD Organisation for Economic Co-operation and Development, 2002<br>Not available; Not applicable.<br>GLP/GEP: no<br>Published: yes | N | N | Public |

[14] Guidance of EFSA: 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

| **Data point** | **Author(s)** | **Year** | **Title**<br>**Company Report No**<br>**Source (where different from company)**<br>**GLP or GEP status**<br>**Published or not** | **Vertebrate study**<br><br>**Y/N** | **Data Protection Claimed**<br><br>**Y/N** | **Owner** |
|---|---|---|---|---|---|---|
| KMA 1.3/04<br>MA B.1.3.1/01 | Schöenfelder, M. | 2006 | The baculovirus preparation SENPV<br>Andermatt Biocontrol AG, CH, not applicable<br>not available<br>GLP/GEP: no<br>Published: no | N | N | ABA |
| KMA 1.3/07<br>MA B.1.3.1/02<br>MA B1.3.2/01 | Jehle, J.A., Lange, M., Wang, H., Hu, Z., Wang, Y., Hauschild, R. | 2006 | Molecular identification and phylogenetic analysis of baculoviruses from lepidoptera.<br>Virology, 346, 180-193<br>Not available; Not applicable.<br>GLP/GEP: no<br>Published: yes | N | N | Public |
| KMA 1.3/03<br>MA B.1.3.2/02 | Rohrmann, G.F. | 2013 | Chapter 1: introduction to the baculoviruses, their taxonomy and evolution<br>Baculovirus Molecular Biology, 3rd edition, 1-24<br>Not available; Not applicable.<br>GLP/GEP: no<br>Published: yes | N | N | Public |
| KMA 1.3/11<br>MA B.1.3.2/03<br>MA B1.3.3/11 | Thèzè, J., Cabodevilla, O., Palma, L., Williams, T., Caballero, P., Herniou, E.A. | 2014 | Genomic diversity in european *Spodoptera exigua* multiple nucleopolyhedrovirus isolates<br>not available, not applicable<br>Journal of General Virology, 95, 2297-2309<br>GLP/GEP: no<br>Published: yes | N | N | - |
| MA B.1.3.2/04 | King, A. M.; Lefkowitz, E.; Adams, M. J.; Carstens, E. B. | 2011 | Baculoviridae. In Virus Taxonomy: Ninth Report of the International Committee on Taxonomyof Viruses; Eds.; Elsevier, 2011; pp. 163–173.<br>GLP/GEP: no<br>Published: yes | N | N | |
| MA B.1.3.2/05 | Yingjian Chen | 2019 | Identification and genomic sequence analysis of a new *Spodoptera exigua* multiple nucleopolyhedrovirus, SeMNPV-QD, isolated from Qingdao, China.<br>Journal of Invertebrate Pathology 160 (2019) 8–17 | N | N | |
| MA B.1.3.2/06 | Thézé, J.; Lopez-Vaamonde, C.; Cory, J.S.; Herniou, E.A., | 2018 | Biodiversity, Evolution and Ecological Specialization of Baculoviruses: A Treasure Trove for Future Applied Research.<br>Viruses 2018, 10, 366.<br>Not available; Not applicable.<br>GLP/GEP: no<br>Published: yes | N | N | Public |

| **Data point** | **Author(s)** | **Year** | **Title**<br>**Company Report No**<br>**Source (where different from company)**<br>**GLP or GEP status**<br>**Published or not** | **Vertebrate study**<br><br>**Y/N** | **Data Protection Claimed**<br><br>**Y/N** | **Owner** |
|---|---|---|---|---|---|---|
| MA B.1.3.3/01 | R. Murillo, M.S. Hussey, and R. D. Possee | 2011 | Evidence for covert baculovirus in 4 fections in a *Spodoptera exigua* laboratory culture | N | N | |
| MA B.1.3.30/02 | Gelernter, W. D. and Federici, B. A. | 1986 | Isolation, identification and determination of virulence of a nuclear polyhedrosis virus from the beet army worm, *Spodoptera exigua* (Lepidoptera: Noctuidae). Environmental Entomology 15, 240-245. | N | N | |
| KMA 1.3/07<br>MA B.1.3.3/03<br>MA B1.1.3.4/01 | Jehle, J., Matt-Schmid, A. | 2007 | comparative restriction analysis of *Spodoptera exigua* nucleopolydrovirus (SPEMNPV) SPEXIT with SEMNPV SPOD-X<br>Andermatt Biocontrol AG, CH, not applicable<br>not available<br>GLP/GEP: no<br>Published: no | N | N | ABA |
| MA B.1.3.3/04<br>B.1.3.3/01 | Jehle, J.A., Lange, M., Wang, H., Hu, Z., Wang, Y., Hauschild, R | 2006 | Molecular identification and phylogenetic analysis of baculoviruses from Lepidoptera<br>not available, not applicable<br>Virology, 346, 180-193<br>GLP/GEP: no<br>Published: yes | N | N | |
| KMA 1.3/05<br>MA B.1.3.3/05 | Jianfeng, Z. | 2005 | the identity of *Spodoptera exigua* nuclear polyhedrosis virus (SENPV) strain<br>Andermatt Biocontrol AG, CH, not applicable<br>not available<br>GLP/GEP: no<br>Published: no | N | N | ABA |
| KMA2.7/03<br>MA B.1.3.3/06 | Heldens, J. G., E. A. Van Strien, A. M. Feldmann, P. Kulcsar, D. Mu~noz, D. J. Leisy, D. Zuidema, R. W. Goldbach, and J. M. Vlak | 1996 | *Spodoptera exigua* multicapsid nucleopolyhedrovirus deletion mutants generated in cell culture lack virulence in vivo. J. Gen. Virol. 77: 3127–3134. | N | N | |

| **Data point** | **Author(s)** | **Year** | **Title**<br>**Company Report No**<br>**Source (where different from company)**<br>**GLP or GEP status**<br>**Published or not** | **Vertebrate study**<br><br>**Y/N** | **Data Protec-tion Claimed**<br><br>**Y/N** | **Owner** |
|---|---|---|---|---|---|---|
| MA B.1.3.3/07 | Caballero, P.; T. Williams Y M. López-Ferber | 2001 | Estructura y clasificación de los baculovirus. pp. 15-46. En: P. Caballero, M. López-Ferber y T. Williams [editores], Los baculovirus y sus aplicaciones como bioinsecticidas en el control biológico de plagas. Phytoma-España, Valencia, España. | N | N | open literature |
| MA B.1.3.3/08 | Lasa, R., Williams, T., Caballero, P | 2008 | Insecticidal Properties and Microbial Contaminants in a *Spodoptera exigua* Multiple Nucleopolyhedrovirus (Baculoviridae) Formulation Stored at Different Temperatures. | N | N | open literature |
| MA B.1.3.3/09 | Sonia Elvira, M. Angeles Ibargutxi, Noelia Gorria, Delia muñoz, Primitivo caballero, and Trevor Williams | 2013 | Insecticidal Characteristics of Two Commercial *Spodoptera exigua* Nucleopolyhedrovirus Strains Produced on Different Host Colonies. J. Econ. Entomol. 106(1): 50-56 | N | N | open literature |
| MA B.1.3.3/10 | Zamora-Avilés | 2018 | Zamora-Avilés, N., Murillo, R., Lasa, R., Pineda, S., Figueroa, J.I., Bravo-Patiño, A., Díaz, O., Corrales, J.L., Martínez, A.M., 2017. Genetic and biological characterization of four nucleopolyhedrovirus isolates collected in Mexico for the control | N | N | open literature |
| KMA 1.3/10<br>MA B.1.3.3/11 | Wang, Q., Bosch, B.-J., Vlak, J.M., van Oers, M.M., Rottier, P.J., van Lent, J.W.M. | 2016 | Budded baculovirus particle structure revisited<br>not available, not applicable<br>Journal of Invertebrate Pathology, 134, 15-22<br>GLP/GEP: no<br>Published: yes | N | N | - |
| KMA 1.3/09<br>MA B.1.3.5/01 | Gueli Alletti, G. | 2018 | Literature review on *Spodoptera exigua* multiple nucleopolyhedrovirus (semnpv: Biological properties<br>Andermatt Biocontrol AG, CH, 356159-MA-02-01<br>GAB Consulting GmbH, Heidelberg, Germany<br>GLP/GEP: no<br>Published: no | N | Y | ABA [15] |

[15] ABA – Andermatt Biocontrol AG