European Commission

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

Spodoptera exigua multicapsid nucleopolyhedrovirus (SeMNPV) strain BV-0004

Active substance data

Volume 3 – Annex B.3 Data on Application

Rapporteur Member State: Spain

April 2020

Version History

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.

[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.3 Data on application

B.3.1 Function

Viral entomopathogen. Biological insecticide. The active ingredient of SPEXIT® is a naturally occurring virus of *Spodoptera exigua*. It is highly selective for larvae of *S. exigua* and therefore harmless to beneficial and other non-target organisms Anonymous (2018).

B.3.2 Field of use envisaged

Control of *S. exigua* in horticulture and home-gardening.
Report KMA 3.2/01 – Anonymous (2018), Spexit insecticide for the biological control of the beet armyworm (*S. exigua*) Not published
Summary: Not applicable, extended summary above

RSM comments: Document KMA 3.3/01 SPEXIT "Insecticide for the biological control of the beet armyworm (*S. exigua*) provide general information and recommendations for use of SPEXIT product. There is no information in KMA 3.2/01 document about the author, neither the date.

B.3.3 Crops or products protected or treated

SeMNPV is intended to be used in pepper, cucumber, watermelon, strawberry, lettuce, carnation and hops (KMA 3.2/01).

Phytochemicals may affect the performance of insecticides in the tritrophic interaction involving an herbivorous insect, a host plant and the pest control agent. The better the host chemistry is surveyed, the better the phytochemical variation leading to possible differences in the pest mortality can be understood. This fact was observed to be an important factor in the management of pest insects. The infectivity of BVs to herbivore insects is affected by phytochemicals, which are ingested during the acquisition of viral inoculum on the foliage of the host plant (Wan *et al*. 2016). This fact was observed to be an important factor in the management of pest insects. Different host plant contained the BV have different chemical compounds, that can affect the infectivity to the target insect. Therefore, it is important to known the host chemistry and the phytochemical variation leading to possible differences in the pest mortality.
Wan *et al.* (2016) tested the effects of different host plant species on the infectivity of SeMNPV to *S. exigua* larvae and detected a significant effect for host plant and virus dose on the corrected mortality and mean time to death. The lowest LD_{50} values were measured for the host plants Chinese cabbage (*Brassica chinensis*), corn (*Zea mays*) and soya bean (*Glycine max*). The highest LD_{50} values were measured for water convolvulus (*Ipomoea aquatica*), collards (*B. oleracea*) and radish (*Raphanus sativus*). Taking those findings into consideration, slight differences in the infectivity of SPEXIT depending on the host plants phytochemicals may be expected.

Reference:	Wan, N.-F., Jiang, J.-X., Li, B. (2016). Effect of host plants on the infectivity of nucleopolyhedrovirus to Spodoptera exigua larvae.
Report No.:	**KMA 3.3/02**
Guideline:	N/A
GLP:	No
Summary:	Previous studies have shown that the infectivity of baculovirus to herbivores is affected by phytochemicals ingested during the acquisition of viral inoculum on the foliage of host plants. Here, we measured the effects of 14 host plant species on the infectivity of *Spodoptera exigua* nucleopolyhedrovirus (SeNPV) to its larvae. The order of the LD_{50} values of SeNPV among the host plants was *Ipomoea aquatica* > *Brassica oleracea* > *Raphanus sativus* > *Amaranthus tricolor* > *Spinacia oleracea* > *Vigna unguiculata* > *Solanum melongena* > *Capsicum annuum* > *Apium graveolens* > *Allium fistulosum* > *Lactuca sativa* > *Brassica chinensis* > *Zea mays* > *Glycine max*, with

	940.1 ± 2.26, 424.0 ± 0.60, 295.2 ± 1.13, 147.3 ± 0.63, 138.6 ± 0.22, 119.9 ± 0.07, 119.8 ± 0.02, 109.2 ± 0.18, 104.8 ± 0.62, 102.1 ± 0.66, 97.9 ± 0.22, 89.9 ± 0.32, 79.0 ± 0.13 and 64.0 ± 0.38 OBs per larva, respectively, and the values of mean time to death of virus-infected larvae were 6.21 ± 0.11, 7.12 ± 0.10, 7.33 ± 0.21, 6.97 ± 0.02, 7.06 ± 0.01, 7.29 ± 0.03, 7.32 ± 0.05, 7.07 ± 0.08, 7.24 ± 0.11, 7.09 ± 0.13, 7.50 ± 0.06, 7.23 ± 0.01, 7.30 ± 0.02 and 7.19 ± 0.07 days, respectively. The mean time to death of larvae decreased with increasing viral dose, and corrected mortality decreased as the larval mean time to death increased. These findings have significance for understanding the effects of host plants on the infectivity of baculovirus to noctuids.
M&M:	Leaf disc method was used, in which aqueous suspenssion of the virus is pippetted onto the leaf discs, and fourty-eight larvae were places.
Test substances:	• **Antagonist:** SeMNPV from field and propageted in third-instar larvae of *S. Exigua* in laboratory. Purified OBs in water suspension. • **Target pathogen***:* Beet armyworm *(S. Exigua)* from field and larvae propagated in laboratory.
tested plants:	**Host plants:** soya bean, lettuce, spinach, collards, radish, Chinese cabbage, onion, corn, eggplant, pepper, water convolvulus, cowpea, celery, amaranthus tricolor. Fourteen different species crops.
Treatments:	Five OBs concentrations: $8.75x10^6$, $1.75x10^6$, $3.5x10^6$, $7\ x10^6$, $1x10^7$, $1.4x10^7$OBs/ml and fourtyeight larvae per plant disc assessed. Three replicate plant per virus concentration and two repetitions per bioassay.
Duration:	24h, until the larvae consumed the entire plant disc. Days until death (ten days).
Results:	Host plant and virus dose had significant effects on the correlated mortality and mean time to death of beet armyworm larvae and that interaction (host plant x virus dose) has a significant effect on the infectivity.

RMS findings: The study clearly showed that host plant species played an important role in mediating the infectivity of the entomopathogen SeMNPV to *S. exigua*, which modified the key aspects of the insect-baculovirus interactions. Eventhough, the conclusion agrees with the applicant: In the case of the SeMNPV intended used lettuces and pepper, only for the $7x\ 10^6$ OBs/ml dose there is a significant difference in larvae percentage of mortality and there is no difference in time death either in LD_{50} and LT_{50}. Virulence and transmission are positively related among genotypes of SeMNPV. These systems clearly demonstrate that the interactions between the insects and the pathogen are highly context dependent. Not only is the outcome a consequence of changes in density and genetic diversity: environmental factors, particularly diet, can have strong impacts on virulence, transmission and host resistance or tolerance.

B.3.4 Method of production and quality control

CONFIDENTIAL information, please refer to confidential document Volumen 4

B.3.5 Information on the occurrence or possible occurrence of the development of resistance of the target organism(s)

In the past, the phenomenon of resistance towards microbial pest control agents has scarcely been investigated. Thus, there is little information on this subject in the published literature. Strategies for resistance management are reviewed and discussed by Roush (1998) who stressed some key indicators of the potential for resistance, including specificity of 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). However, BVs cause a polyorganotropic disease so that the risk of developing resistance is rather low.

Concerns about resistance in *S.exigua* against chemical insecticides and also against *Bacillus thuringiensis* toxins increased in the last years, but no reports are available on resistance of *S.exigua* towards SeMNPV. Only recently, the first case of reduced susceptibility of a host towards a baculovirus was observed against CpGV in some codling moth (*Cydia pomonella*) populations (Fritsch *et al*., 2005).

In the related noctuid moth *Helicoverpa armigera*, resistance to chemical pesticides did not reduce susceptibility towards HearNPV (Vijaykumar *et al*., 2003a), but on the other hand, HearNPV infection increases the susceptibility of *H. armigera* to chemical insecticides (Vijaykumar *et al*., 2003b).

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. Thus 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.

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. In 2003–06, a systematic survey of 13 organic orchards in southern Germany where several populations of the codling moth (*Cydia pomonella)* were found that were highly resistant to the *Cydia pomonella* granulovirus (CpGV), the efficacy of CpGV had been reported as unsatisfactory.

No similar incidents are known for SeMNPV, but 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 study was included by the RMS to support the assessment of the possible development of resistance in BVs of the target organisms. Monitoring and resistance management would be necessary.

Reference:	Asser-Kaiser, S., Fritsch, E., Undorf-Spahn, K., Kienzle, J., Eberle, K.E., Gund, N.A., Reineke, A., Zebitz, C. P. W., Heckel, D.G., HuberJehle, J. A., (2007). Rapid Emergence of Baculovirus Resistance in Codling Moth Due to Dominant, Sex-Linked Inheritance. Science, 317, 1916-1918
Guideline:	N/A
GLP:	No
Summary:	Insect-specific baculoviruses are increasingly used as biological control agents of lepidopteran pests in agriculture and forestry, and they have been previously regarded as robust to resistance development by the insects. However, in more than a dozen cases of field resistance of the codling moth *Cydia pomonella* to commercially applied *C. pomonella granulovirus* (CpGV) in German orchards, resistance ratios exceed 1000. The rapid emergence of resistance is facilitated by sex-linkage and concentration dependent dominance of the major resistance gene and genetic uniformity of the virus. When the gene is fixed, resistance levels approach 100,000-fold. Our findings highlight the need for development of resistance management strategies for baculoviruses.

RMS comments

Study is included to support the assessment of 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 (Clem and Passarelli 2013). SeMNPV diversity in low-density host populations can be surprisingly high. The spatial structure of the virus can be also hierarchical, with the virus from families and then populations being more similar than those on different strains. This is likely to be an underestimate of the variation within the virus population. 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. What maintains the high level of host and pathogen diversity in these systems, however, remains a question.

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. Virto *et al*. 2016, observed that distinct genotypes of SeMNPV differ in their insecticidal properties and can be associated with horizontal or vertical routes of transmission. Thereby, research described, that novel combinations of horizontally and vertically transmitted genotypes in an alphabaculovirus-based insecticide could provide immediate pest control and contribute to transgenerational pest suppression of *S. exigua*

larval populations in agro-ecosystems (Virto *et al*. 2016, 2017). Also the study conducted by Caballero *et al*. (2009) described the insecticidal potential of mixtures of certain SeMNPV genotypes and measured a promising field efficacy.

Sublethal infections by SeMNPV are common in field populations of *S. exigua*. In laboratory assays, a significant reduction in pupal weight, adult emergence, fecundity, and fertility (egg hatch) and a significant increase in larval developmental time and duration of the preoviposition period, was found in *S. exigua* with a sublehtal infection. Such covert infections were found to persist for at least five generations. Individuals from a persistently infected experimental population were significantly more susceptible to SeMNPV OB inoculum than individuals without a sublethal infection. It was concluded that sublethal infections may benefit pest control programs, given that insects that do not die from an overt infection after consuming contaminated food have a high probability of reduced reproduction and that pest control level is improved from one generation to the next (Cabodevilla *et al*. 2011a). Further studies conducted by the same research group, detected the prevalence of sublethal infections in *S. exigua* populations and measured a positive infection in 16.1 % of the surveyed individuals (Cabodevilla *et al*. 2011b). Also in this study, it was observed that sublethally infected insects are more susceptible to infection than healthy insects. Vertical transmission, were the baculovirus are transferred from one generation to the other, has been suggested as a pathogen survival strategy when opportunities for horizontal transmission are highly restricted (Virto *et al*. 2016).

B.3.6 Methods to prevent loss of virulence of seed stock of the micro-organism

Confidential information - see Vol 4, section C.1.4.

Lasa *et al*. (2008) surveyed the insecticidal properties SeMNPV formulation (phosphate-buffered saline, pH 6.5, with 5 % glycerol (vol:vol) and 0.15 % sorbic acid (wt: vol)) stored at different temperatures: - 20 ºC, 4 °C and 25 ºC. The initial aerobic counts were thereby measured after 17 h incubation at 37 °C. Significant changes in OB concentrations over an 18 months storage period were only observed in the 25 °C treatment. The estimated LD_{50} values of OBs stored at 25 °C increased by >16.7-fold over the storage period compared with newly formulated OBs, whereas LD_{50} values were not greatly affected by storage at 4 or - 20 °C. The processes that lead to decreased baculovirus infectivity during storage is not completely understood yet. It is assumed that deterioration may be due hydrolysis and autoxidation as result to exposure to oxygen derived from lipids that are present in insect cadaver remains (in the case of an *in vivo* production). This may result in proteolysis or the production of free radicals and superoxides, which are capable to disrupt the nucleic acid structure. Lasa *et al*. (2008) assumed an enormous DNA degradation in occulated virions during storage at 25 °C. Whereby DNA purified from OBs that had been stored refrigerated of frozen did not suffer degradation. The researchers concluded that OB formulation with bacteriostatic or antioxidant additives, storage and distribution in refrigerated conditions, allows a SeMNPV shelf life of >18 months and that the method of extraction, purification, formulation and the storage condition have an impact on nucleopolyhedrovirus preparations.

Reference:	Lasa, R., Williams, T., Caballero, P. (2008) Insecticidal Properties and Microbial Contaminants in a *Spodoptera exigua* Multiple Nucleopolyhedrovirus (Baculoviridae) Formulation Stored at Different Temperatures.
Report No.:	**KMA 3.6/01**
Guideline:	N/A
GLP:	No
Summary:	The *S. exigua* (Hübner) multiple nucleopolyhedrovirus (SeMNPV) is currently being tested as a biological insecticide for use in greenhouse crops in southern Spain. We performed a study in which semipurified SeMNPV occlusion bodies (OBs) were formulated in phosphate-buffered saline, pH 6.5, with 5% (vol:vol) glycerol and 0.15% (wt:vol) sorbic acid, and they were stored at -20, 4, or 25°C during 18 mo. Initial aerobic counts (±SE) averaged 1.4 (±0.17) × 10^7 colony-forming units/ml after 17-h incubation at 37°C. Aerobic counts of microorganisms that contaminated OB formulations stored at 25°C decreased markedly over the period of the study, whereas only small decreases were observed in counts from OBs stored at 4 or -20°C. The principal microbial contaminants of OB suspensions were *Enterococcus* spp., Enterobacteriaceae, and yeasts. Potential human pathogens (*Salmonella*, *Shigella*, and *Vibrio* species) were not detected, and populations of *Staphylococcus aureus* and *Bacillus cereus* were extremely low. Compared with newly formulated OBs, the estimated LD_{50} values of OBs stored at

	25_C increased by _16,666-fold over the 18 mo of storage, whereas LD_{50} values were not greatly affected by storage at 4 or -20°C. Significant changes over time in OB concentrations were only observed in the 25°C treatment. Complete degradation of viral DNA was observed at 25°C but not in refrigerated or frozen OBs. We conclude that OB formulation with bacteriostatic or antioxidant additives, together with storage and distribution in refrigerated conditions, will likely result in an SeMNPV biopesticide shelf life that exceeds 18 mo.
M&M:	• **Microbial contaminants:** Aerobic Counts on Microbial Contaminants and microbial analysis. • **Physical and molecular stability of OBs:** viral DNA and restriction endonuclease analysis. • **Eficacy:** Bioassays on Insecticidal Activity.
Test substances: Duration:	• **Antagonist:** Spanish isolate of SeMNPV from field and propageted in fifth-instar larvae of S. Exigua in laboratory. Purified OBs in water suspension. • **Target pathogen:** Beet armyworm (S. Exigua) mass production of laboratory colony of larvae propagated in laboratory. • **Formulate of SeMNPV:** phosphate-buffered saline (PBS), pH 6.5, 0.15% (wt:vol) sorbic acid and 5% (vol:vol) glycerol. 35 samples of each sample batch. Storage at different temperaturs (25, 4 and -20ºC). • 18 month of storage
Results:	**Microbial contaminants:** density of microorganims during storage differ significantly on temperature. The formulated OBs suspension contained an average of 1.9 10^8 CFU/g, composed of 50% aerobes and 50% anaerobes. **Physical and molecular stability of OBs:** There were significant difference in the DNA extracted form OBs stored at different temperatures. It is not possible to visualize DNA in agarose gel in samples stored at 25ºC, the DNA was seriously degraded. DNA extracted from OBs stored at 4 and -20ºC presented the characteristc profile of this endonuclease. Eventhough, there a small smearilling observed in DNA extracted from OBs stored at 4ºC compared to DNA stored at -20ºC, suggested a degree of DNA degradation. **Eficacy: Bioassays on Insecticidal Activity:** LD_{50} of the formulated OBs suspenssion was 7.9 Obs per larvae. The effect of temperature was highly sgnificant: LD_{50} at 25ºC increase by 2.5 fold and 273-fold at 6 and 12 months, at 18 month exceeded the highest rate of of OBs. Insecticidal activity does not altered after 18 months at 4ºC, whereas storage at -20ºC has a significant decrease in the LD_{50} value.

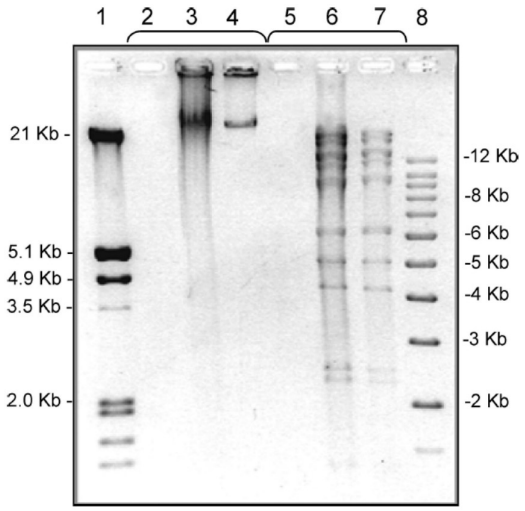

Figure MA3.6-1: Viral DNA and Restriction Endonuclease Analysis.Agarose gel electrophoresis of DNA isolated from SeMNPV OBs (pooled samples from three repetitions) after 18 months of storage at 25, 4 and -20ºC (lane 2,3,4) and BglII restriction analysis of the DNA samples after endonuclease restriction (lane 5,6,7). Molecular size markers were lambda DNA/Ecoli RI+HinIII marker (lane1) and 1-Kb DNA ladder (lane 8) (Lasa *et al*., 2008).

RMS comments: Even the formulate product is not the same; it is very similar, and useful to analyse the storage behaviour of SeMNPV. The study examined changes in microbial loads during storage of a formulated product containing the microbiostatic adjuvant sorbic acid. 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. SeMNPV products storage and distribution in refrigerated conditions would favour a product shelf life of over 18 months.

Significant differences were observed in the genomic DNA extracted from OBs stored at different temperature conditions (**Figure MA3.6-1**). It was not possible to visualize DNA in agarose gels in the OB sample stored at 25ºC, indicating of DNA was seriously degraded and could not be recovered. In contrast, DNA extracted from OBs stored at 4 or -20ºC presented the characteristic pattern for this REM. However, the smearing observed in DNA extracted from OBs stored at 4ºC suggests a degree of DNA degradation compared with DNA from OBs stored at -20ºC (lanes 6 and 7).

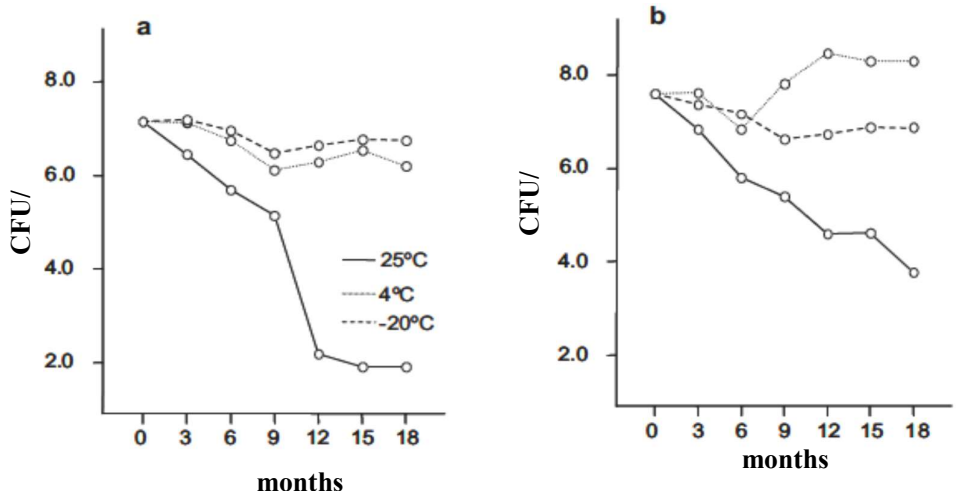

Figure MA3.6-2 Changes in aerobic CFU/ml SeMNPV OB suspenssions stored during 18 months at different temperaturas. Colony growth was scored after incubation on solid media plates at 37ºC after 17h of incubation (a) and reincubated until 48h at 28ºC (b). (Lasa *et al*., 2008).

The density of microorganisms during storage differed significantly depending on the temperature and duration of storage for microbes counted at 17 h, or when reincubated for 48 h (**Figure MA3.6-2)**. Additional incubation until 72 h did not result in a significant increase in CFU counts, compared with those at 48 h, and so 72 h counts are not considered further. Aerobic CFU counts of microorganisms that contaminated the OB formulation stored at 25ºC showed a steady decrease during the entire period of the study. Storage at-20ºCwith periodic thawing and refreezing resulted in a small decrease in CFU counts during the 18 months period. Storage at 4ºC resulted in a similar pattern of CFU counts as that observed at -20ºC.

The results of the anaerobic analyses indicated that the presence of anaerobes or micro-aerobes are not likely to give cause for concern in baculovirus-based biopesticides. No vertebrate pathogens such as, Vibrio spp., Shigella spp., and Salmonella spp., were detected in SeMNPV samples. Vertebrate pathogens have not been found in other BVs production systems, except for the sporadic occurrence of B. cereus (Podgwaite *et al*. 1983) A healthy insect colony and hygiene of workers are necessary to minimize contaminants like B. cereus, for which some toxin-producing strains can cause food poisoning if present at high concentrations (10^5-10^6 CFU/g) or S. aureus, which commonly inhabits the human skin surface and nasopharynx, but can cause abscesses of the skin and eyes (Garcia-Lara *et al*. 2005). The activity of SeMNPV stored under refrigerated or frozen conditions was equivalent that of no stored formulated material. The insecticidal activity was compromised after 6 months of storage at 25ºC and eliminated after 18 months storage at this temperature.

The LD_{50} value of the formulated OB suspension in *S. exigua* second instars was estimated at 7.9 OBs per larva. The effect of temperature on insecticidal activity after 18 months of storage was highly significant. The estimated LD_{50} value of OBs stored at 25ºC increased by 2.5-fold and 273-fold after 6 and 12 months of storage, respectively. After 18 months at 25ºC, the LD_{50} exceeded the highest rate of OBs used in the bioassay, representing >16,666-fold loss of insecticidal

activity. In contrast, insecticidal activity was not significantly altered after 18 month of storage at 4ºC, whereas storage at 20ºC with periodic thawing to evaluate biological activity followed by refreezing resulted in a significant but small decrease in the LD_{50} value, compared with material bioassayed after 6 months of storage.

Overall conclusion, 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. 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.

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.

On the other hand, the activity of SeMNPV stored under refrigerated or frozen conditions was comparable that of newly formulated material.

B.3.10 References relied on

Literature search:

The literature search carried out by the applicant performed in accordance with the recommendations in the EFSA (2011)[13] 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 DialogTM. The data requirement "Biological properties of the micro-organism" was covered using a focused search encompassing baculoviruses 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 3.3/01.

Regarding Section 3, two references were identified as relevant (in Point MA 3.3, MA 3.6).

From peer reviewed open literature, no fundamental new findings on crops or products protected or treated were identified.

ABA – Andermatt Biocontrol AG

[13] 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 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 3.2/01 MA B.3.1/01 MA B.3.2/01	Anonymous	2018a	SPEXIT Insecticide for the biological control of the beet armyworm (Spodoptera exigua) Andermatt Biocontrol AG, CH, not available not available GLP/GEP: no Published: no	N	Y		ABA
KMA 3.3/02 MA B.3.3/01	Wan, N.-F., Jiang, J.-X., Li, B.	2016	Effect of host plants on the infectivity of nucleopolyhedrovirus to Spodoptera exigua larvae not available, not applicable Journal of Applied Entomology, 140, 636-644 GLP/GEP: no Published: yes	N	N	not protected	-
KMA 3.5/01 MA B.3.5/01	Roush, R.T.	1998	Strategies for Resistance Management not available, not applicable GLP/GEP: no Published: yes	N	N	not protected	-
KMA 3.5/02 MA B.3.5/02	Fritsch, E., Undorf-Spahn, K., Kienzle, J., Zebitz, C.P.W., Huber, J.	2005	Apfelwickler-Granulosevirus: Erste Hinweise auf Unterschiede in der Empfindlichkeit lokaler Apfelwickler-Populationen not available, not applicable Nachrichtenblatt des Deutschen Pflanzenschutzdienstes, 57, 29-34 GLP/GEP: no Published: yes	N	N	not protected	-
KMA 3.5/03 MA B.3.5/03	Vijaykumar, K.B.K., Fakrudin, B.	2003a	Effectiveness of Helicoverpa armigera Nuclear Polyhedrosis Virus Against Insecticide Resistant Strains of Helicoverpa armigera (Hubner) (Lepidoptera: Noctuidae) not available, not applicable Resistant Pest Managemant Newsletter, 13, 27-28 GLP/GEP: no Published: yes	N	N	not protected	-
KMA 3.5/04 MA B.3.5/04	Vijaykumar, K.B.K., Fakrudin, B.	2003b	Effect of Nuclear Polyhedrosis Virus Infection on the Insecticide Susceptibility of Heliothis armigera Larvae not available, not applicable Resistant Pest Managemant Newsletter, 13, 28-30 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
MA B.3.5/05	Asser-kaiser asser-kaiser, s., fritsch, e., undorf-spahn, k., kienzle, j., eberle, k.e., gund, n.a., reineke, a., zebitz, c. P. W., heckel, d.g., huberjehle, j. A.,	2007	Rapid Emergence of Baculovirus Resistance in Codling Moth Due to Dominant, Sex-Linked Inheritance. Science, 317, 1916-1918	N	N	not protected	-
MA B.3.5/06	Clem, R. J., and A. L. Passarelli	2013	BACULOVIRUSES: SOPHISTICATED PATHOGENS OF INSECTS. PloS Pathogens 9:e1003729	N	N	not protected	-
MA B.3.5/07	Virto, C., Navarro, D., del Mar Tellez, M., Williams, T., Murillo, R., Caballero, P.	2016	Mixtures of vertically and horizontally transmitted variants of Spodoptera exigua multiple nucleopolyhedrovirs (SeMNPV) as the basis for biological insecticides not available, not applicable IOBC/wprs Bulletin, 113, 131-135 GLP/GEP: no Published: yes	N	N	not protected	-
MA B.3.5/08	Virto, C., Williams, T., Navarro, D., Tellez, M. M., Murillo, R., and Caballero, P.	2017	Can mixtures of horizontally and vertically transmitted nucleopolyhedrovirus genotypes be effective for biological control of Spodoptera exigua? J. Pest Sci. 90, 331–343. doi: 10.1007/s10340-016-0 743-x	N	N	not protected	-
MA B.3.5/09	Caballero, P., Murillo, R., Munoz, D., Williams, T.	2009	El nucleopoliedrovirus de Spodoptera exigua (Lepidoptera: Noctuidae) como bioplaguicida: análisis de avances recientes en España not available, not applicable REvista Colombiana de Entomología, 35(2), 105-115 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**
MA B.3.5/10	Cabodevilla, O., Villar, E., Virto, C., Murillo, R., Williams, T., and Caballero, P.	2011a	Intra- and intergenerational persistence of an insect nucleopolyhedrovirus: Adverse effects of sublethal disease on host development, reproduction, and susceptibility to superinfection. Applied and Environmental Microbiology, 77(9), 2954–2960. GLP/GEP: no Published: yes	N	N	not protected	-
MA B.3.5/11	Cabodevilla, O., Ibañez, I., Simón, O., Murillo, R., Caballero, P., Williams, T.	2011b	Occlusion body pathogenicity, virulence and productivity traits vary with transmission strategy in a nucleopolyhedrovirus not available, not applicable Biological Control, 56, 184-192 GLP/GEP: no Published: yes	N	N	not protected	-
KMA 3.6/01 MA B.3.6/01	Lasa, R., Williams, T., Caballero, P.	2008	Insecticidal Properties and Microbial Contaminants in a Spodoptera exigua Multiple Nucleopolyhedrovirus (Baculoviridae) Formulation Stored at Different Temperatures not available, not applicable Journal of Economic Entomology, 101(1), 42-49 GLP/GEP: no Published: yes	N	N	not protected	-
MA B.3.6/02	Podgwaite, J.D., Dubois, N.R., Reardon, R.C. and, Witcosky, J	1993	Retarding outbreak of low-density gypsy moth (Lepidoptera: Lymantriidae) populations with aerial applications of Gypchek and Bacillus thuringiensis. Journal of Economic Entomology 86, 730-734.	N	N	not protected	-
MA B.3.6/03	Garcia-Lara, J., A. J. Needham, and S. J. Foster	2005	Invertebrates as animal models for Staphylococcus aureus pathogenesis: a window into host-pathogen interaction. FEMS Immunol. Med. Microbiol. 43: 311Ð323.	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 3.7/01 MA B.3.7/01	OECD	2002	Consensus document on information used in the assessment of environmental applications involving baculovirus not available, not applicable ENV/JM/MONO, 1, 1-90 GLP/GEP: no Published: yes	N	N	not protected	-
KMA 3.7/02 MA B.3.7/02	Frommer, W., Ager, B., Archer, G., Collins, C.H., Donikian, R., Frontali, C., Hamp, S., Houwink, E.H., Küenzi, M.T., Krämer, P., Lagast, H., Lund, S., Mahler, J.L., Normand-Plessier, F., Sargeant, K., Tuijnenburg Muijs, G., Vranch, S.P., Werner, R.G.	1989	Safety precautions for handling microorganisms of different risk classes not available, not applicable Applied Microbiology and Biotechnology, 30, 541-552 GLP/GEP: no Published: yes	N	N	not protected	-
KMA 3.7/03 MA B.3.8/01	Anonymous	2018b	Safety Data Sheet SPEXIT Andermatt Biocontrol AG, CH, not available not available GLP/GEP: no Published: no	N	Y		ABA

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KMA 3.3/01	Gueli Alletti, G.	2018	Literature review on Spodoptera exigua multiple nucleopolyhedrovirus (SeMNPV: Biological properties Andermatt Biocontrol AG, CH, 356159-MA-02-01 GAB Consulting GmbH, Heidelberg, Germany GLP/GEP: no Published: no	N	Y		ABA