European Commission

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

Spodoptera exigua multicapsid nucleopolyhedrovirus (SeMNPV)

Product data: SPEXIT

Volume 3 – Annex B.10 Summary and evaluation of environmental impact

Rapporteur Member State: Spain

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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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B.10 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.

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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.*

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:

- 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.*

PPP (product name/code):	SPEXIT	Formulation type:	SC
Active Substance: (SeMNPV)	*Spodoptera exigua* multicapsid nucleopolyhedrovirus	Conc. of a.s.:	3.75×10^{12} OBs/L
Applicant:	Andermatt Biocontrol GmbH	professional use	☒
Zone(s):	EU	non professional use	☒
Safener:	n.a.	Conc. of safener:	n.a.
Synergist:	n.a.	Conc. of synergist:	n.a.
Verified by RMS:	yes		

Table MP B. 10.1. Summary of critical Good Agricultural Praxis for SPEXIT

n.a. Not applicable

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

B. 10 SUMMARY AND EVALUATION OF ENVIRONMENTAL IMPACT

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

B. 10.1 Distribution and fate of SPEXIT

B. 10.1.1 Fate and behaviour in soil

Based on available information derived from published literature on BVs the environmental fate and population dynamics of this BV can be summarized as follows:

BVs persist immobile in the soil for a fairly long period. Accumulation can only occur following mass multiplication in the lepidopteran host. BVs have also been detected in areas where they had never been artificially applied. It has been established that the virus titre in soil decreases rapidly after field treatment and only small numbers of BVs are found in the following year. Experience with different BVs has shown that the viruses are inactivated or mineralised in soil by a number of parameters including UV light, pH and soil bacteria. Nucleopolyhedroviruses are increasingly inactivated at lower soil pH values. It is conjectured that at low pH values the free carbonate available in the soil inactivates the virus.

The nature of the bioinsecticide SPEXIT does not allow application of soil degradation studies and calculation of time weighted average concentrations, as employed for chemical substances, since 'degradation' or decline of populations of microorganisms does not follow first order kinetics of degradation.

B10.1.1 Predicted environmental population density in soil (PECsoil)

In order to perform a risk assessment for non-target organisms, the actual concentration of SPEXIT is calculated for soil, based on a maximum application rate of 3.6 L SPEXIT/ha (0.2 L/ha maximum field dose rate x 18 applications, i.e. max. 3 per generation, max. 6 generations, assuming as a worst case that no degradation occurs between applications).

For risk assessment, the resultant load of SPEXIT is related to the top 5 cm of soil to achieve the highest theoretical soil concentration, although in practice incorporation will occur within the top 10 to 15 cm of soil.

Guideline:	• Guidance document on Persistence in soil (9188/VI/97 rev 8) EU Commission (2000) • Soil persistence models and EU registration (FOCUS, 1997)
Material and methods:	
Substance considered:	3.6 L SPEXIT/ha (= 1.35×10^{13} OB/ha or 4.18 kg/ha MPCP, density: 1.16 g/ml)
maximum field dose	3.6 L SPEXIT/ha ~1.35×10^{13} OB/ha
Crop interception:	0%
Degradation	No degradation assumed (worst case), 1000 days (default).
Mixing depth:	For calculation of initial PECsoil: 5 cm.
Bulk density:	1.5g/cm^3
Endpoints calculated:	- Initial PECsoil after one application:

$$\text{Initial PECsoil (mg/kg)} = \frac{\text{A (g/ha)}}{100 \times \text{d (cm)} \times \rho \text{ (g/cm}^3\text{)}}$$

where: A = effective application rate (after adjusting for any crop interception)
d = depth of soil layer (5 cm)

ρ = soil bulk density (1.5 g/cm3)

- Accumulated PECsoil after 10 years of applications

PECsoil = Initial PECsoil × e^{-kt}

where: $k = \ln 2/DT_{50}$ and $DT_{50}=0$

PEC_{soil} = accumulated application rate / soil mass

= 4.18 kg/ha / 750000 kg = 5.57 mg/kg soil

According to the PEC_{soil} calculation for EXPEXIT

PEC_{soil} =Initial PEC_{soil}	**5.57 mg/kg soil~1.8 x 10^7 OB/kg dry weight soil**

B10.1.2 Fate and behaviour in water

B10.1.2 .1 Surface water

Spodoptera exigua MNPV consists of OB, which do not dissolve in water. In general, however, it may be stated that the OB will be completely mineralised by bacterial action in water and sediment. The active ingredient is destroyed, among other factors, by the action of UV light. It may thus be stated that *Spodoptera exigua* MNPV is inactivated under natural conditions, including water. No multiplication of SeMNPV can occur as the hosts, *S. exigua* larvae, do not occur in water.

Following the Guidance Document on Aquatic Ecotoxicology (SANCO/3268/2001), the maximum drift rate is 6.26 % (vegetables, ornamental plants, berries), of the applied amount at a distance of 3 m to surface waters. No degradation between the applications is assumed.

Applic. rate kg/ha	**Rate mg/m²**	**Distance (m)**	**Drift (%)[2)]**	**Amount of drift**		**Initial PEC_{sw} (µg/L)**	
				g/ha	**mg/m²**	**1 m**	**30 cm**
4.18[1)]	418	3	6.26	261.668	26.167	26.17	87.14[3)]

[1)] equivalent to 1.35 x 10^{13} OB/ha

[2)] according to BBA spray drift data from 27. March 2006, www.bba.bund.de

[3)] equivalent to 2.83 x 10^5 OB/L

Table B10.1.2.1-1 Calculation (vegetables, ornamental plants, berries) of the predicted environmental concentration of SPEXIT in lentic water bodies (PECsw)

B10.1.2.2 Ground water

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

experiments in glass columns revealed high absorptiveness in the top organic soil layers and very little capacity to leach to lower layers.

B10.1.2.3 Drinking water

Drinking water quality is monitored by screening for microbial indicator species. Potential interference with the analytical systems for the control of the quality of drinking water according to Council Directive 98/83/EC needs to be addressed. For drinking water coliforms or *E. coli*, enterococci, and *Pseudomonas aeruginosa* are monitored. Monitoring of these bacteria is accomplished by cultivating them on appropriate media. SeMNPV as a virus is not able to proliferate in the absence of its host. Therefore, any interference with analytical systems for drinking water can be excluded.

B10.1.3 Fate and behaviour in air

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

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

An investigation of photochemical-oxidative degradation in air is of no relevance in view of the volatility characteristics of the virus.

B10 2 Identification of non-target species at risk and extent of their exposure

B10.2.1 Effects on birds

The experience that BVs present no risk for non-target species has been confirmed by numerous literature studies cited in Annex MA Point 8.1. SeMNPV is highly specific and only has an effect on larvae of *Spodoptera exigua*.

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

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

B10.2.2 Effects on fish

An acute toxicity study on rainbow trout was conducted with the product Granupom containing *Cydia pomonella* GV. Neither significant mortality nor sublethal effects were observed at all concentrations below the nominal concentration level of 100 mg Granupom/L over 96 h. The LC_{50} value at 96 h was estimated to be > 100 mg Granupom/L (> 2.04 x 10^9 OB/L at a density of 1080 g/L), the highest concentration tested. Similar results are expected with SPEXIT.

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

B10.2.3 Effects on freshwater invertebrates

An acute toxicity study on *Daphnia magna* was conducted with the product Granupom containing *Cydia pomonella* GV. At 48 h Granupom did not show any toxic effects on *Daphnia magna*. The EC_{50} is greater than 100 mg/L, the NOEC is 100 mg/L (2.04 x 10^9 OB/L at a density of 1080 g/L), the highest concentration tested, with a probability of more than 99.9 %. Similar results are expected with SPEXIT. Based on the predicted environmental concentration (PEC_{sw}), calculated as 188.36µg SPEXIT/L (6.13 x 10^5 OB/L) previously, margin of safety (MOS; corresponding to TER) for freshwater invertebrates is derived from the EC_{50} value. Based on the submitted data on effects on aquatic organisms and the intended use in fields and glasshouses, the calculated margin of safety values are high and it is anticipated that the potential risk posed to *Spodoptera exigua* multicapsid nucleopolyhedrovirus (SeMNPV) to daphnids is very low and acceptable.

B10.2.4 Effects on single cell algae

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

B10.2.5 Effects on single cell algae

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

B10.2.6 Effects on terrestrial vertebrates other than fish

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

B10.2.6.1 Exposure

Birds and mammals are typically exposed to dry spray deposits on their food items following the dilution and via drinking water following spraying of the formulated product. During spraying, much of the formulation constituents are likely to be lost by volatilisation. Therefore, where oral exposure is the main route of exposure, toxicity data for the active substance are used in preference to data from tests with the formulated material. Exposure via dermal and inhalation routes is considered unlikely, since at the time of application and for a short period thereafter, most wild

mammals will leave the immediate vicinity of spray operations in response to the human disturbance. Mammals may be exposed directly and indirectly via the ingestion of sprayed plant parts and via infected arthropods, respectively.

The potential exposure of mammals to SeMNPV was estimated following GAP directed applications of the product in the different uses at maximum application rates.

For risk assessment for effects on mammals the 'European Food Safety Authority Guidance Document on Risk Assessment for Birds and Mammals' (EFSA Guidance document 2009)[13] is available. However, this document in first line is compiled for the risk assessment of chemical substances. Therefore, the risk assessment approach is not feasible for microbial substances as not only biological parameters of the mammals go into calculations but also chemical properties, like K_{oc} values from the test item, 90th percentile residue values that come from a database for chemicals.

For the exposure via drinking water a risk assessment in accordance to SANCO 4145/2000[14] is presented, which is considered more appropriate and is considered to represent a worst-case.

B10.2.6.2 Exposure via drinking water

Risk assessment to drinking water is performed in accordance with SANCO 4145/2000[2]. Species that frequent open water bodies are able to ingest spray deposits of active substances that reach water for example via spray drift from treated fields.

In some situations, some species may obtain all their daily water demand directly from puddles of spray liquid or reservoirs held in the axils of leaves. This situation can be considered as worst case. The exposure density can be calculated from the dilution used to prepare the product for spraying. Analysis has shown that initial densities in such sources are in the range 5 – 20 % of the sprayed concentration, therefore a dilution factor of 5 is applied for the risk assessment.

The risk of SeMNPV to mammals was assessed from margin of safety (MOS; corresponding to TER). Calculation of the exposure via water can be considered worst case. The density in the water is directly related to the spray application. In the drinking water risk assessment for mammals the SeMNPV specific endpoints in OB/kg bw were used for the calculations. The resulting MOS values indicate that no adverse effects in mammals are to be expected due to exposure to "contaminated" drinking water following GAP directed use of SPEXIT.

B. 10.2.7 Effects on bees

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

It is appropriate to assume that the corresponding values for SeMNPV will also be $>3.5\times10^7$OB/bee for oral LD_{50}/72h and $> 4.4 \times 10^7$ OB/bee for contact LD_{50}/48h. With a density of 1.16 kg/L and a content of 3.75 ×x 10^{12} OB/L, these contents of virus occlusion bodies correspond to 10.8 mg/bee and 13.6 mg/bee of SPEXIT, respectively.

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

[13] European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12): 1438. [139 pp.].

[14] European Commision, Health & Consumer Protection Directory, Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC, SANCO/4145/2000 - final, 25 September 2002

Even all considerations mentioned related to the specificity of BV, the RMS consider convenient to include at least one specific study with bees, due to the relevance of these species, that can be exposure to the dissolved OB in the active substance treated area (worst-case assumptions). There is no information on the short-term and long-term espouse on the ingestion by bees of *S. exigua* virus from SPEXIT spraying. No acute end-points are available and no studies on bees were submitted on infectivity from Andermatt Biocontrol GmbH.

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

B. 10.2.8 Effects on arthropods other than bees

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

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

The acute toxicity of the formulation CpGV SC (= Granupom) to the aphid parasitoid *Aphidius rhopalosiphi* (Hymenoptera, Braconidae) was determined *in vitro*. Based on the results of this study it is assumed that Granupom will have no effects on *Aphidius rhopalosiphi* under field conditions up to the tested application rate of 360 mL Granupom/ha. Considering a content of 2.2 x 10^{13} viable granules/L for Granupom and a density of 1080 g/L this results in an NOEC value for Granupom of 0.389 kg Granupom/ha, which is equivalent to 7.9 x 10^{12} viable granules/ha. It is appropriate to assume that the corresponding values for SeMNPV will also be 7.9 x 10^{12} OB/ha, corresponding to 2.43 kg SPEXIT/ha.

The acute toxicity of the formulation CpGV SC (= Granupom) to the predatory mite *Typhlodromus pyri* (Acarina, Phytoseiidae) was determined in a laboratory study. Based on the results of this study it is assumed that Granupom will have no effects on *Typhlodromus pyri* under field conditions up to the tested application rate of 360 mL Granupom/ha. Considering a content of 2.2 x 10^{13} viable granules/L for Granupom and a density of 1080 g/L this results in an NOEC value for Granupom of 0.389 kg Granupom/ha, which is equivalent to 7.9 x 10^{12} viable granules/ha. It is appropriate to assume that the corresponding values for SeMNPV will also be 7.9 x 10^{12} OB/ha, corresponding to 2.43 kg SPEXIT/ha.

The acute toxicity of the formulation CpGV SC (= Granupom) to the ground beetle *Poecilus cupreus* (Coleoptera, Carabidae) was determined in a laboratory study. Granupom will not affect *Poecilus cupreus* under field conditions up to the tested application rate of 450mL/ha. Considering a content of 2.2 x 10^{13} viable granules/L for Granupom and a density of 1080 g/L this corresponds to an NOEC value for Granupom of 0.486 kg Granupom/ha, which is equivalent to >9.9 x 10^{12} viable granules/ha. It is appropriate to assume that the corresponding values for SeMNPV will also be 9.9 x 10^{12} OB/ha, corresponding to 3.06 kg SPEXIT/ha.

The calculation of HQ values as used for chemicals (application rate/LD_{50}) is generally regarded as less feasible for risk assessments with microbial biocontrol agents (mBCAs) because dose-response relationships are rarely observed in cases of pathogenic effects (OECD 2012[15]).

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

[15] OECD Guidance to the Environmental Safety Evaluation of Microbial Biocontrol Agents, Series on Pesticides No. 67, ENV/JM/MONO(2012)1

If mortality or signs of intoxication are observed in the tests the evaluation must include a calculation of toxicity/exposure ratios based on the quotient of the ER 50 value (effective rate) and the estimated exposure.

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

B. 10.2.8.2 Risk assessment for other arthropods

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

B. 10.2.9 Effects on earthworms

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

B. 10.2.10 Effects on soil microorganisms

An assessment of the side effects of CpGV on the activity of the soil microflora was conducted by Wachter (1998b, please refer to Annex MP 10.6) using the formulation CpGV SC (= Granupom). The impact on soil respiration of soil type 1 and soil type 2 is considered as negligible (< 15 % deviation) even at the 10-x dosage of the highest recommended Granupom application rate (5 L/ha: 5.94×10^{14} granules/ha). Considering the similarity in formulation of the products Granupom and SPEXIT and in the included BVs CpGV and SeMNPV, these results can be transferred to SPEXIT. Thus, no adverse effects on soil microflora are expected after application of SPEXIT at a total application rate of 18 applications: 3.6 L/ha: 1.35×10^{13} OB/ha.

B10.3 Identification of precautions necessary to minimize environmental contamination and to protect non-target species

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

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

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

B10.4 Reference list

No references are submitted in this section.