European Commission

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

Spodoptera exigua multicapsid nucleopolyhedrovirus (SeMNPV)

Active subtance data

Volume 3 – Annex B.6 Toxicology

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

Table of contents

B.6. EFFECTS ON HUMAN HEALTH

This dossier is submitted by Andermatt Biocontrol AG, Switzerland, for the approval of *Spodoptera exigua* multicapsid nucleopolyhedrovirus (SeMNPV) as microbial pest control agent under the Regulation (EC) 1107/2009 of the European Parliament.

Active substances are approved for maximum period of 10 years under Directive 91/414/EEC[1]. The active substance SeMNPV was under programme of renewal Regulation EU 686/2012 (AIR-III programme[2]). According to draft working document AIR III renewal programme SANCO/2012/11284[3], *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[4] 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.

SeMNPV is a baculovirus. Baculoviruses belong to the family Baculoviridae, which are arthropod-specific, enveloped viruses with a circular double-stranded DNA genome.

SeMNPV acts highly specific against larvae of the beet armyworm, *Spodoptera exigua*. It is supposed not to have any harmful effects on organisms not belonging to the genus *Spodoptera*. With regard to safety considerations it is important to note that SeMNPV and the whole family of Baculoviridae are naturally present in our environment. As baculoviruses are closely associated with their host, occurrence of SeMNPV is linked to the presence of the host, *Spodoptera exigua*. Therefore, their application in pest control means only a fluctuation of the virus titre in the biotope of the pest insect. The experience that baculoviruses present no risk for the environment has been confirmed by numerous studies. *Spodoptera exigua* multicapsid nucleopolyhedrovirus (SeMNPV) is a naturally occurring virus.

[1] *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.*
[2] *Programme of renewal Regulation EU 686/2012 (AIR-III programme).*
[3] *SANCO/2012/11284 –rev. 22, December 2018. Draft working document AIR III renewal programme.*
[4] *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.*

B.6.1. TIER I

B.6.1.1. Basic information

Baculoviruses 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 baculoviruses is restricted to one or a few species of the genus or family of the host where they were originally isolated.

Due to their host specificity, baculoviruses do not affect other organisms like vertebrates, arthropods other than their host species, micro-organisms, or plants. Baculoviruses do not produce any metabolites at all.

During the biphasic replication cycle of baculoviruses, two distinct viral phenotypes are formed: Occluded virions (ODV) and budded virions (BV) 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 nucleocapsids 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 family Baculoviridae is divided into the four genera: Alphabaculovirus, Betabaculovirus, Gammabaculovirus and Deltabaculovirus. In the past, the family was divided into Nucleopolyhedroviruses (NPV) and Granulovirus (GV) and the classification was based on the morphology of the occlusion body (OB), nowadays the classification is based on genome phylogeny. 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. 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 baculovirus core genes, which are shared among alpha-, beta-, gamma- and deltabaculoviruses. The conserved genes are involved in 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.

Baculoviruses are named according to the OB morphology and the host where the virus was isolated from. Recent phylogenetic analysis, based on 30 core genes, showed that the Baculoviridae family colnsists of four monophyletic groups (Alphabaculovirus, Betabaculovirus, Gammabaculovirus and Deltabaculovirus), which is also linked to the insect orders of the corresponding hosts and on the morphology. The Alphabaculoviruses are further divided into two subgroups, group I and group II (Jehle, 2006). Lepidopteran-specific baculoviruses are divided into Alpha- and Betabaculoviruses encompassing the NPVs and GVs, respectively. Hymenoptera-specific baculoviruses (NPVs) represents Gammabaculoviruses and Dipteran-specific baculoviruses (GVs) Deltabaculoviruses (Rohrmann, 2013). There are substantial differences in the morphology (e.g. occlusion body) observed among the different Baculoviridae genera. According to sequence and phylogenetic analysis, Lepidopteran NPVs were further split into Group I and Group II (including SeMNPV) whereby the subdivision is correlated with the employment of two different envelope glycoproteins, i.e. GP64 (group I) and F (group II) (Lung, 2002).

The fact that baculovirus 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, namely polyhedrin/granulin, late expression factor (lef) 8 and lef-9. (Jehle, 2006).
Spodoptera exigua multicapsid nucleopolyhedrovirus (SeMNPV) is the valid name of the virus species. In the literature, the term *Spodoptera exigua* nucleopolyhedrovirus (SeNPV) is also used and refers to the same species. Other baculoviruses isolated from Spodoptera species, namely S. litura NPV (SpltNPV), S. terricola NPV (SpteNPV), and S. littoralis NPV (SpliNPV) were also previously classified in the group II NPVs. Today, they are all classified to genera Alphabaculoviruses. These species are very closely related to each other, but are more distantly related to SeMNPV (Jehle, 2006).

Basic information was compiled by Krieg (1976). "Baculoviruses, being highly specific agents, are not supposed to have any harmful effect on personnel in research or industrial mass production of viral insecticides. The same is true for field application of such viruses as well as for consumption of virus-treated crop. With regard to safety problems it is important to note that baculoviruses are naturally present in our environment. Therefore, their application in pest control means only a fluctuation of the virus titre in the biotope of the pest insect. The experience that contact of baculoviruses with man or animals does not involve any risk for their health has been confirmed by special tests. For registration purposes, safety tests are carried out according to WHO standards. In addition, tests on the potential infectivity have been made on mammalian cell cultures as well as on mutagenicity, teratogenicity and oncogenicity on animals, all with negative results".

Both nucleopolyhedroviruses (NPV) and granuloviruses (GV) form occlusion bodies (OB). Whereas the OB of GV contain only a single nucleocapsid with the viral envelope, NPV OB can harbour a single (SPNV) or multiple nucleocapsid (MNPV) per virion. The OB protects the virus against damaging environmental conditions and allows the virions to remain viable for many years. The matrix protein of GV, granulin, is genetically and serologically closely related to the NPV matrix protein polyhedrin. The host ranges of both GV and NPV are exclusively restricted to arthropods. No member of this family is toxic or infective to plants and vertebrates. Considerations on toxicology and human health are discussed in OECD consensus paper No. 20 (OECD 2002) with the conclusion that "the use of baculoviruses is safe".

The *Spodoptera exigua* multicapsid nucleopolyhedrovirus (SeMNPV) does not affect any organism except larvae of few species within the genus *Spodoptera*. No toxins are produced. Toxic metabolism or degradation products do not occur.

A literature search according to EFSA guidance (2011)[5] was conducted in January 2018 covering the last 10 years (Seehase, 2018). It contained search terms relevant for the human health risk assessment. The literature research was carried out using the search-engine ProQuest Dialog™ and the selected databases AGRICOLA, AGRIS, MEDLINE; BIOSIS® Previews, CAB Abstracts, Embase®, and SCISEARCH. After rapid assessment based on title and abstract, three references were submitted to a detailed assessment of the full text documents. No references were considered relevant for the evaluation of effects on human health.

In this health evaluation of SeMNPV, reference is made to data obtained with other baculovirus species belonging either to the genus Granulovirus (GV) or to the genus Nucleopolyhedrovirus (NPV). The applicant states that because of the close relationships within the family *Baculoviridae*, results and findings from studies with NPV are considered applicable to SeMNPV and may be used for risk assessment purposes. Both NPV and GV form occlusion bodies (OB). [Note: Sometimes, the synonymous term "inclusion body/bodies" is still in use.] The OB protects the virus against damaging environmental conditions and allows the virions to remain viable for many years. However, in spite of the general similarities between GV and NPV, some differences do exist and must be taken into consideration (see Table **6.1.1.1-1**). Whereas the OB of GV, e.g., contain only a single nucleocapsid with the viral envelope, NPV OB can harbour a single (SPNV) or multiple nucleocapsids (MNPV) per virion. However, the matrix protein of GV, granulin, is genetically and serologically closely related to the NPV matrix protein polyhedron. For the applicant, this similarity is considered more important for risk assessment than the number of nucleocapsides since it influences the infectivity and antigenicity or immunogenicity very much.

However, in the opinion of the RMS, studies with SeMNPV should be carried out.

[5] 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

Table 6.1.1-1: Comparison of Nucleopolyhedroviruses (NPV) and Granuloviruses (GV) according to the International Committee on Taxonomy of Viruses database

Criteria	NPV	GV
Viral protein	Polyhedrin	Granulin
Shape of the crystalline protein matrix	Polyhedral	Ovicylindral
Size of occlusion bodies (OB)	0.15 – 15 µm	0.13 – 0.5 µm
Number of virion per OB	One or several	One
Nature of virions in OB	Single nucleocapsid (S) or multiple nucleocapsids (M) within a single viral envelope	Single nucleocapsid (S)
Envelope projections	Terminal surface projections	Surface projections
Size of complete genome (nucleotides)	90,000 – 165,000	100,000 – 180,000

From the recent literature search, no references were identified reporting medical cases of SeMNPV. In addition, the Occupational health statement submitted under point B.6.1.1.2 did not report any incidences related to adverse health effects.

B.6.1.1.1. Medical data

Viruses are obligate intracellular parasites, i.e., they can only multiply inside living cells. The basic consideration on their safe use for plant protection purposes should address the ability of a certain virus species or strain to infect other organisms than the target species that is intended to be controlled.

Baculoviruses are naturally occurring pathogens of arthropods. Baculoviruses, especially Granuloviruses, have a narrow host range and are strictly host-specific to certain arthropod species. This host specificity has been demonstrated *in vivo* and *in vitro* in numerous mammalian cell lines. In fact, these viruses do not replicate in vertebrate cells. An infection of cells or animals is confined to the target species because of the molecular biological mode of action of the baculovirus. While baculoviruses may enter mammalian cells, the species-specific nature of the infection is dependent on the promoter of the baculovirus, which is active only in Lepidoptera (Gronowski, 1999). *Spodoptera exigua* multicapsid nucleopolyhedrovirus (SeMNPV) does not affect any organism except larvae of few species within the genus *Spodoptera*. Because of its nature as a virus, a toxin is not produced. Likewise, toxic metabolites or degradation products do not occur.

Baculoviruses are part of our natural environment and have been used for biological insect control for more than 100 years. There is no evidence that these viruses have ever caused any disease process in humans or other mammals. This assessment is supported by a long and complete safety record comprising safety tests of more than 51 entomopathogenic viruses, including more than 30 baculoviruses, in mammals (extensively reviewed by Ignoffo, 1973; Burges, 1980; Gröner, 1986).

B.6.1.1.2. Medical surveillance on manufacturing plant personnel

A current occupational health statement from Andermatt Biocontrol AG was submitted for evaluation of SeMNPV under Regulation (EC) No 1107/2009. Andermatt Biocontrol AG is manufacturing SeMNPV since 2006 and have not reported any incidents related to adverse health effects, abnormalities or casualties to persons engaged in production and handling of the microbial products (Zingg, 2018).

B.6.1.1.3. Sensitisation/ allergenicity observations, if appropriate

No case of sensitization or allergenic responses or respiratory troubles of workers were observed during mass production of different baculoviruses including SeMNPV at Andermatt Biocontrol AG (Andermatt, 2006a & b).

No cases of sensitisation have been reported in a current occupational health statement (Zingg, 2018), and no cases of sensitisation or allergic reactions have been reported in a comprehensive literature search (Seehase, 2018).

Nevertheless, according to Regulation (EC) 283/2013, all microorganisms should be regarded as potencial sensitisers.

B.6.1.1.4. Direct observations e.g. clinical cases

For SeMNPV no practical data and information exist on the recognition of symptoms of infection or pathogenicity. Since SeMNPV lacks any infectivity to man or animals, no symptoms of pathogenicity can be developed upon exposure.

Studies in human volunteers

For SeMNPV, data is not available. However, a study in human volunteers was reported with another baculovirus, the cotton bollworm (*Heliothis zea*) NPV (Heimpel and Buchanan, 1967). Nine men and one woman (between 24 and 60 years old), volunteering as test subjects, each consumed a total dose of about 5.82×10^9 polyhedra per person that was split over an exposure period of five days. The virus was administered in gelatine capsules once a day after breakfast. Four other men and two women (age range from 21 to 42 years) who served as controls were administered sterile insect protein from the same preparation that was obtained by separating the polyhedra before but further details on their treatment were not given. It seems that all these volunteers (whose names were made public in the journal article) had been recruited from the staff of the Entomology Research Division of the U.S. Department of Agriculture. The study was conducted under medical supervision. Complete physical examinations and a variety of laboratory tests (haematology, urinalysis and a limited range of blood clinical chemistry parameters) were performed prior to and at 9 and 30 days after first dosing. These examinations failed to show any significant change in the general health condition of the participating individuals neither in the test nor in the control group and did not reveal any differences between the two groups.

RMS comment and conclusions

It is concluded that no adverse reactions in Andermatt Biocontrol AG personnel involved in production and handling were reported as a result of exposure to SeMNPV.

No cases of sensitisation have been reported in the current occupational health statement. The study is considered additional information.

B.6.1.2 Basic studies

RMS Introductory comment

Numerous studies were carried out in different kinds of animals; however, none were conducted with SeMNPV, but, as the applicant considers that all baculoviruses have the same behaviour, the use of more animals in new studies should be avoided following the Directive 2010/63/UE. In the opinion of the RMS, studies with SeMNPV should be carried out.

B.6.1.2.1. Sensitization

Available methods for testing dermal or respiratory sensitisation are not suitable for testing microorganisms. Nevertheless, a skin sensitisation study with guinea pigs was conducted with *Spodoptera littoralis* NPV ([redacted] 1976a). Eight guinea pigs were challenged with a 1% suspension of a preparation containing 6×10^{10} OB/g. Another skin sensitisation study ([redacted] 1986) and one respiration sensitisation study ([redacted] 1992) were performed with *Cydia pomonella* GV, and both cases did not show any allergic response of guinea pigs.

These three studies are summarized below.

B.6.1.2.1/01 Skin sensitization assay 01

Reference:	[redacted] (1976a)
Report:	Testing for sensitising properties of nuclear polyhedrosis virus (NPV) in guinea pigs by the method of Landsteiner Unpublished Report No. 148/76
Guideline(s):	Not stated, method of Landsteiner
Deviations:	Not applicable
GLP:	No
Acceptability:	Yes

Material and Methods

Test Item	
Designation	*Spodoptera littoralis* NPV
Purity	6.0×10^{10} p/g
Test System	
Species	Male Pirbright-White guinea pigs
Body weight	200 – 300 g
Number	8
Study Design and Methods	
In-life dates	16.02.1976 to 22.03.1976
Exposure	Intracutaneous
Vehicle	1 % suspension in physiological saline
Post exposure observation:	14 days
Experimental treatment	The virus material was injected intracutaneously into the shaved flank of 8 animals on ten days over a period of three weeks (at 3-days intervals). Another 8 animals served as control. Animals were then left untreated for two weeks before challenge treatment by injection of the test substance. The injection dose for the first treatment was 0.05 ml per animal, and for the other nine treatments 0.1 ml per animal. After the final injection, the animals were left untreated for 14 days. A challenge treatment was carried out by infection of 0.05 ml of the virus suspension. The dermal reactions were evaluated after 24 and 48 hours.
Observations	Animals were observed for weight gains.

Results and Conclusions

A. Results

The 10 intracutaneous injections of virus suspension caused erythema and pustule formation at the injection sites of all 8 treated animals. After 5 to 7 days healing took place after scab formation and cicatrisation. The erythema and pustule formation can be accounted for by bacterial contamination of the virus material. The body weight gains of all animals during the study and observation periods were regular (see **Table 6.1.2-1**). The challenge treatment with 0.05 ml virus suspension elicited slight erythema within 24 hours at the injection sites of all 8 pre-treated guinea pigs. Pustules also formed here after 48 to 72 hours. Eight non-sensitised control animals showed the same reaction after injection of 0.05 ml of the 1 % virus suspension.

Table 6.1.2-1: Body weight gains of 8 male guinea pigs sensitised for 3 weeks with nuclear polyhedrosis virus NPV, and of 8 control animals

Group	n	Date	Mean body weight [g]	SE
Control animals	8	16.02.1976	240.13	15.625
		23.02.1976	307.38	14.798
		01.03.1976	370.25	21.212
		08.03.1976	442.50	21.673
		15.03.1976	507.75	20.659
		22.03.1976	576.75	25.047
Sensitised animals	8	16.02.1976	242.38	7.247
		23.02.1976	313.38	7.981
		01.03.1976	367.38	14.121
		08.03.1976	433.13	24.554
		15.03.1976	501.00	32.758
		22.03.1976	571.25	36.644

B. Conclusion

Testing of nuclear polyhedrosis virus NPV yielded no indications of allergenic properties in a sensitisation study by the method of Landsteiner.

RMS comment and conclusions

The report concludes that Spodoptera littoralis NPV, not the baculovirus notified, does not produce hypersensitivity in guinea pigs. Although the study has not been conducted with SeMNPV, according to the OECD Consensus Document (ENV / JM / MONO (2002) 1), baculovirus species are extremely host-specific and only occur in arthropods. Baculoviruses are not infective for mammals and replication does not occur in mammalian cells. Therefore, the study carried out with another baculovirus could be extrapolated to the one notified. Nevertheless, following the Regulation (EC) 283/2013, all microorganisms should be considered potential sensitisers.

B.6.1.2.1/02 Skin sensitization assay 02

Reference:	[redacted] (1986)
Report:	Hoe 083311 OI LC08 A101, Testing for sensitising properties in Pirbright-White guinea Pigs by the method of Landsteiner Unpublished Report No. ID 86.1373; A55528
Guideline(s):	Not stated, method of Landsteiner
Deviations:	Not applicable
GLP:	Yes
Acceptability:	Yes

Material and Methods

Test Item	
Designation	Granupom (Formulation containing Cydia pomonella GV)
Characteristics	clear liquid

Batch no.	Hoe 083311 OI LC08 A101
Expiration date	Not stated
Purity	2.2×10^{10} granules CpGV/mL
Test System	
Species	female Pirbright-White guinea pigs [strain Hoe, DHPK (SPF Lac)]
Source	[redacted] SPF breeding colony
Number	20
Acclimatisation period	At least 5 days
Study Design and Methods	
Exposure	Intradermal injection, topical treatment
Vehicle	Physiological saline
Post exposure observation:	17 days
Experimental treatment	A non-guideline conform study following the method of Landsteiner was conducted under GLP to assess dermal sensitisation of Granupom in Pirbright-White guinea pigs. The test substance was intradermally injected on 10 subsequent days into the shaved left front flank of 10 animals. Another 10 animals served as controls and were treated with physiological saline only. On day 34, the first challenge treatment was applied: control and test substance group received identical (probably topical but not clearly stated in the report) treatment with Granupom on the shaved right front flank. On day 36, a similar second challenge treatment was applied to the left rear flank. Macroscopic examination of the skin for erythema and oedema was conducted on day 37.
Observations	Animals were observed for general clinical signs, body weight gains, and signs of irritation (scores according to Draize) at 24 and 48h post the induction and challenge phase.

Results and Conclusions

A. Results

During the induction phase, very slight to well-defined erythema and occasionally very slight oedema were observed at animals of the test substance group suggesting slight irritation by the test material when injected into the skin (**Table 6.1.2-2**). However, scores after the 1st and 2nd challenge treatment revealed no significant differences in skin reactions between the test and control groups (**Table 6.1.2-3).**

Table 6.1.2-2: Skin examinations after 10th treatment during induction phase

Group	n	Type of irritation	Scoring[a)] after 10th treatment during induction phase										Mean
			1	2	3	4	5	6	7	8	9	10	
Control	10	Erythema[b)]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0
		Edema	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0
Test substance	10	Erythema[b)]	1.0	2.0	2.0	2.0	1.0	2.0	2.0	2.0	2.0	1.0	1.7
		Edema	0.0	0.0	0.0	1.0	0.0	1.0	1.0	1.0	0.0	0.0	0.4

a) according to Draize (0-4)
b) over small areas only

Table 6.1.2-3: Skin examinations 24 and 48 hours after 1st and 2nd challenge treatment

Group	n	Type of irritation	1st challenge scoring [a)] after		2nd challenge scoring after	
			24 h	48 h	24 h	48 h
Control	10	Erythema[b)]	2/1/2/2/2/2/2/2/1/2	1/1/1/1/1/1/1/1/0/1	2/2/2/2/2/2/2/2/1/2	0/0/1/1/0/0/0/1/1/0
		Edema	1/0/1/1/1/1/1/1/0/1	0/0/0/0/0/0/0/0/0/0	1/1/1/1/1/1/1/1/0/1	0/0/0/0/0/0/0/0/0/0
Test substance	10	Erythema[b)]	2/1/1/1/2/2/2/2/1/2	1/1/1/1/1/1/1/1/1/1	2/2/1/1/2/2/2/2/2/2	0/1/1/0/1/0/1/1/1/1
		Edema	1/1/0/0/1/1/1/1/1/1	0/0/0/0/0/0/0/0/0/0	1/1/0/0/1/1/1/1/1/1	0/0/0/0/0/0/0/0/0/0

a) according to Draize (0-4)
b) over small areas only

B. Conclusion

No skin reactions were observed in Granupom treated animals. Body weight and behaviour were not affected by the treatment. The formulation Granupom did not produce skin sensitisation in guinea pigs.

RMS comment and conclusions

The report concludes that Cydia pomonella GV, not the baculovirus notified, does not produce skin sensitisation in guinea pigs. Although the study has not been conducted with SeMNPV, according to the OECD Consensus Document (ENV / JM / MONO (2002) 1), baculovirus species are extremely host-specific and only occur in arthropods. Baculoviruses are not infective for mammals and replication does not occur in mammalian cells. Therefore, the study carried out with another baculovirus could be extrapolated to the one notified. Nevertheless, following the Regulation (EC) 283/2013, all microorganisms should be considered potential sensitisers.

B.6.1.2.1/03 Respiratory sensitization assay 03

Reference:	[redacted] (1992)
Report:	Hoe 083311; water miscible suspension concentrate; 2.2×10^{13} Vir./L (Code Hoe 083311 00 SC13 A102) - Testing for respiratory sensitisation in the male and female Pirbright-White guinea pig [redacted]

	Unpublished Report no. 90.0689; A46999, TOX2003-1148.
Guideline(s):	No OECD Guideline applicable; method was favoured by the European Discussion Group of Inhalation Toxicologists (EDIT) and refers to Botham et al. 1989, Toxicology Letters 47, pp. 25-39
Deviations:	No
GLP:	Yes
Acceptability:	Additional information

Material and Methods

Test Item	
Designation	Hoe 083311 (Granupom, *Cydia pomonella*)
Characteristics	water miscible suspension concentrate
Batch no.	C0130 M051
Purity	2.2×10^{10} granules/mL
Test System	
Species	SPF Pirbright White guinea pigs, Hoe DHPK(SPFLac)
Source	[redacted] SPF breeding colony
Number	4 male & 4 female
Study Design and Methods	
Vehicle	Saline
Post exposure observation:	21 days
Experimental treatment	Induction was carried out at day 1 by intradermal injection of 0.1 mL Granupom (12 % solution in isotonic saline). Animals were challenged at day 22 by nose-only aerosol inhalation of approx. 35 mg Granupom/m^3 air (mean median aerodynamic diameter 1.2 µm, geometric standard deviation 1.6) for a period of 15 min. Control animals received isotonic saline only and were similarly exposed to the aerosol as animals from the test group.
Observations	Observations were made on behaviour, clinical signs, primary dermal and respiratory irritation (changes in respiratory rate, tidal volume, minute volume). At termination, gross pathology was also performed.

Results and Conclusions

A. Results

The intradermal injection of the 12 % formulation caused slight erythema and eschar formation. No clinical signs were observed in animals of the control group. The body weight gains were not impaired. Questionable up to slight changes in the respiratory pattern were observed in the control group as well as in the test group. No significant differences were evident between the animals of both groups. Autopsy revealed no macroscopically visible abnormalities.

B. Conclusion

With the formulation Granupom no evidence for sensitisation following intradermal induction and inhalative challenge was obtained.

According to Regulation (EC) 283/2013, the available methods for testing dermal sensitization are not suitable for testing microorganisms as they do not penetrate the skin. Therefore, all microorganisms need to be labelled

with a warning phrase "Microorganisms may have the potential to provoke sensitizing reactions". However, this phrase is not justified for viruses for the following reasons:

- Viruses do not produce metabolites which might be sensitising.
- No signs of sensitisation or allergenicity have been reported in an occupational health report since 2006. There are no published reports on sensitisation induced by SeMNPV.
- Generally, for viral species currently approved in the EU, positive reports on sensitisation are absent.
- As there are no appropriate test methods, it is impossible to demonstrate absence of sensitisation potential.

Evaluators therefore strongly rely on published literature, where very little reports on sensitisation caused by species used for plant protection are found. Reports on sensitisation caused by microbials are mostly restricted to moulds, often in combination with moisture in buildings. This is also confirmed by the EFSA External report "Literature search and data collection on risk assessment for human health for microorganisms used as plant protection products" (Hackl et al. 2015) and a review by Martel et al (2010).

RMS comments and conclusion

The report concludes that Cydia pomonella GV, not the baculovirus notified, does not produce respiratory sensitisation in guinea pigs.There are not validated methods to assess the respiratory sensitization, therefore, this study is considered additional information.

B.6.1.2.2. Acute toxicity, pathogenicity and infectiveness

The available data confirm that baculoviruses do not infect vertebrates and can be considered non-pathogenic and non-toxic. The available studies and publications are reported separately for the different routes of administration

B.6.1.2.2.1. Acute oral toxicity, pathogenicity and infectiveness

General remark: Toxicological studies performed with the formulated products for other baculoviruses are considered applicable and relevant with regard to the evaluation of the active substance, since concentrations of all inert ingredients of the formulated product are without health risk. It should be brought to mind that the formulated product contains huge numbers of "active ingredient", namely 3.75×10^{12} OB SeMNPV/L. The formulated product with its improved physical properties and certainly enough viruses therefore should have only advantages in recognizing potential effects. As contrary to chemical compounds the number of active viruses is responsible for the potential effect and not the volume or weight of the "virus-water" suspension, limit-tests are occasionally conducted with several billion inclusion bodies/kg bw but not necessarily with the minimum weight of the test substance (2000 mg/kg bw) as it is required for chemicals.

B.6.1.2.2.1/01 Acute oral toxicity assay 01

Reference:	[redacted] et al. (1976 b)
Report:	Tolerance testing with nuclear polyhedrosis virus after single oral or intravenous administration to male and female rats Unpublished Report No. 488/76
Guideline(s):	Not stated
Deviations:	Not applicable
GLP:	No
Acceptability:	The study is considered additional information

Material and Methods

Test Item	
Designation	Suspension (in desalted water) of nuclear polyhedrosis virus (NPV) of *Prodenia litura* with a biological activity of 6×10^8 PIB per 10 mg dry substance. The test substance was prepared by infecting 3rd to 4th instar *P. litura* larvae with *P. litura* NPV. Dead larvae were kept for 2 weeks at room temperature until they had putrefied. The resulting suspension was filtered through a cotton cloth, the filtrate was centrifuged at 2000 g, suspended in a small amount of water, and air-dried. Contamination of the virus material with microbial contaminants, insect residues, or metabolites cannot be excluded. This powder was suspended in desalted water and administered to the test animals.
Expiration date	Not stated
Purity	6.0×10^8 p / 10 mg
Test System	
Species	SPF Wistar rats (WISKf – SPF 71)
Source	Breeding colony of [redacted]
Number	20 males and 20 females
Acclimatisation period	8 days
Study Design and Methods	
Exposure	Acute oral and intravenous
Post exposure observation:	21 days
Experimental treatment	A suspension of nuclear polyhedrosis virus (NPV) was administrated once i.v. to rats in a dose of 10 mg/kg (corresponding to a biological activity of 6×10^8 polyhedra inclusion bodies = PIB) or a single oral dose of 50 mg/kg (corresponding to a biological activity of 3×10^9 PIB), after which the animals were kept under observation for 21 days. The animals selected for examination were GT 1287 – 1296/75. Organs were removed in accordance with Dissection schedule III were examined histologically. The following values were determined in the same numbers of animals on the day before treatment and on study day 21 (= day of dissection): blood glucose values by enzymatic method in glucose analyser by method of Beckmann (mmol/l) and SGPT by method of Karmen (mU/ml serum). The control consisted of three groups, one receiving physiological saline by intravenous injection, the other by oral administration. The third group remained untreated. Haematology and clinical chemistry parameters were determined during the study in addition to body weights, food consumption and body temperatures. Body temperature of all animals was measured twice daily throughout the study using a second thermometer (measurement of resistance in semi-conductors) to permit recognition of a possible pryogenic effect of the virus suspension Post mortem examinations were carried out in all of the test animals. To determine the time-dependence of any pathological or anatomical changes, two animals of either sex from each series were killed under Nembutal anaesthesia and dissected on day 3, 7 and 14 after treatment. The remaining animals were autopsied on day 21 after treatment. In these rats, the heart, lungs, liver, kidneys, prostate / uterus, adenal glands, testes / ovaries,

	thyroid gland and pituitary were weighted. From ten males form Series I (nuclear polyhedorsis virus i.v.), which were killed on day 21 after treatment, the following organs and tissues were examines microscopically: heart, lungs, liver, adrenal gland, kidney, brain, testes, epididymides, prostate, pancreas, pituitary, thyroid gland, bone marrow, stomach, intestine, urinary bladder, optic nerve, retina, lymph nodes.
Observations	Observations were done on mortality, clinical organs, body weights, food consumption, body temperatures, haematological and clinical chemistry parameters, gross pathology and histopathology of 18 organs. In addition, daily observations of behaviour and general health condition and continuous measurements of food consumption were varied out.

Results and Conclusions

A. Results

The test animals showed no changes in behaviour or adverse effects on general health condition. Body weight gains and food consumption remained unaffected by treatment with NPV. No effect of NPV treatment on body temperature could be detected. It may be stated very confidentially that there were no changes in organ weights due to NPV.

B. Conclusion

The organs of the rats yielded no evidence of alterations due to NPV.

RMS comment and conclusions

Although the study has not been conducted with SeMNPV, according to the OECD Consensus Document (ENV / JM / MONO (2002) 1), baculovirus species are extremely host-specific and only occur in arthropods. Baculoviruses are not infective for mammals and replication does not occur in mammalian cells. On the other hand, the study should have performed at limit test dose level of 2 000 mg/kg but it was carried out with 50 mg/kg bw dose level. Therefore, the study is considered additional information.

B.6.1.2.2.1/02 Acute oral toxicity assay 02

Reference:	[redacted] (1980)
Report:	Tolerance testing of the AcNPV nuclear polyhedrosis virus following single-dose administration to SPF Wistar rats. (Note: This is the English translation of the original report in German language that is also available to the RMS.) Doc.nos. A55550 (English translation) and A37218 (German original). [redacted] Unpublished report no. 234/80. Dates of experimental work: Nov. 20, 1978 to Dec. 18, 1978; TOX2003-1149.
Guideline(s):	Not applicable
Deviations:	Not applicable
GLP:	No. When the study was performed, GLP was not compulsory. However, it is stated in the report that the GLP regulations that were proposed and discussed at that time had been followed. The experiment was regularly inspected by the QAU.
Acceptability:	Yes

Executive summary

No signs of toxicity, pathogenicity or infectivity have been detected upon single oral exposure to AcNPV. CpGV is of low toxicity based on the LD_{50} in male and female rats.

Material and Methods

Test Item	
Designation	*Autographa californica* Nucleopolyhedrovirus (AcNPV) was replicated in *Spodoptera frugiperda* cells to give a total amount of test material of 1250 mg containing 4×10^{10} polyhedra inclusion bodies (PIB)[6]. Immediately prior to dosing, the test material was suspended in 80 mL high-purity sterilised water. This suspension was administered by oral gavage. The application volume was 10 mL/kg bw, corresponding to a nominal dose of 5×10^9 PIB/kg bw
Test System	
Species	SPF Wistar rats (WISKf – SPF 71)
Source	Breeding colony of [redacted]
Number	20 male and 20 female
Study Design and Methods	
Exposure	Single oral gavage
Vehicle	Not applicable
Post exposure observation:	21 days
Experimental treatment	The application volume was 10 mL/kg bw, corresponding to a nominal dose of 5×10^9 PIB/kg bw. Based on individual bodyweights (145 – 156 g in male rats and 148 -158 g in females), a mean dose level of about 0.725 - 0.780×10^9 PIB for male rats and of $0.740 - 0.790 \times 10^9$ PIB for females was achieved. An untreated control group of the same size (20 per sex) received 10 mL sterilised water/kg bw only. Dosing was followed by a post-observation period of up to 21 days. At study termination on day 21 post dosing, all the surviving rats were killed and subjected to gross pathological examination. Adrenals, gonads, heart, kidneys, liver, lungs, pituitary, prostate and thyroid were weighed. Histopathology was performed on a small number of animals (3 per group and sex) only and comprised the following organs and tissues: duodenum, fat, heart, kidney, liver, lung, lymph nodes (bronchial, cervical, and mesenterial), spleen, and salivary glands.
Observations	Daily observations on behaviour and general health condition were accompanied by determination of body weight (weekly) and food consumption (continuously) and by weekly examinations for neurological symptoms, eye opacities, lesions of the oral mucosa and disturbances of dental growth. Body temperature was measured even twice daily on weekdays. Haematology (parameters: erythrocytes, leucocytes, haematocrit, haemoglobin, prothrombin time, thrombocytes, reticulocytes, coagulation time, differential blood count, Heinz bodies, MCV, MCH, MCHC) was performed prior to treatment and after 14 or 21 days on five animals per sex and group. Limited examination for clinical chemistry parameters (glucose, serum GPT) were carried out before virus administration and on day 21 in those animals that were also employed for haematology. Animals were sacrificed at various dates during the observation period. It is stated in the report that organ and serum from these

[6] *Please note The terms "inclusion bodies" and "occlusion bodies" may be considered in this section as synonyms. Nowadays, "occlusion body/bodies" is preferably used.*

	rats were submitted for virological examination but, unfortunately, the results are not given.

Results and Conclusion

A. Results

Neither mortalities nor clinical signs of a pathogenic response were observed that could be attributed to AcNPV administration (**Table 6.1.2.2.1**). The only death occurring during the course of the study (a male rat from the treatment group that died on day 3 after dosing) was due to an injury during rectal temperature measurement. Behaviour, general health condition, body weight gain and food consumption showed no differences as compared to the control group. Measurements of body temperature showed no abnormalities.

The selected haematology and clinical chemistry parameters remained within the range of normal biological variation and were comparable between the groups. Determination of the absolute and relative organ weights revealed no significant differences between virus-treated and control animals. The macroscopic and microscopic examinations yielded no indications that administration of AcNPV could have caused pathological findings.

Table 6.1.2.2.1: Acute oral toxicity of AcNPV to the rat

Dose	Males		Females	
	Mortality	Time of death	Mortality	Time of death
Untreated	0/20	-	0/20	-
5×10^9 PIB [a] per kg bw	1/20 [b]	DAT 3	0/20	-

[a] polyhedra inclusion bodies
[b] death due to an injury to the rectum during temperature measurement

Oral LD_{50} (combined) > 5×10^9 PIB AcNPV/kg bw

B. Conclusion

Following single oral administration of 5×10^9 PIB AcNPV/kg bw equal to an individual dose of between 0.7 and 0.8×10^9 PIB to rats, no evidence of adverse reactions was obtained that might be indicative of pathogenicity or toxicity. From this study, infectivity cannot be assessed because the results of virological examination were not submitted, however, based on the available knowledge on baculoviruses, it is not likely that experimental AcNPV administration will result in colonisation and replication in rats or other mammals.

No additional information was identified in the literature search covering the last 10 years and focusing on toxicity or pathogenicity of baculovirus to humans and mammals.

RMS comment and conclusions

Although the study has not been conducted with SeMNPV, according to the OECD Consensus Document (ENV / JM / MONO (2002) 1), baculovirus species are extremely host-specific and only occur in arthropods. Baculoviruses are not infective for mammals and replication does not occur in mammalian cells. Therefore, the study carried out with another baculovirus could be extrapolated to the one notified.

B.6.1.2.2.1/03 Other acute oral toxicity assays

The acute oral toxicity (gastric intubation) of 5.8×10^{10} and of 1.8×10^{11} PIB/kg bw of *Orgyia pseudotsugata* NPV to the rat (corresponding to the 98-fold and 307-fold field rate/ha) did not cause deaths. No signs of systemic toxicity/pathogenicity were observed. After autopsy no treatment-related effects were noted (Martignoni, 1978).

Acute oral assays on rats and rabbits with *Neodiprion lecontei* NPV and on rats with *N. sertifer* NPV had no detectable harmful effects (Cunningham & Entwistle, 1981).

Pieris rapae GV was administered at 50 mg/kg bw as a single oral dose to pigs, cows, lambs, chickens, rabbits and mice. None showed ill effects and growth was normal during the 2-3 months that followed (Xuebao, 1982).

B.6.1.2.2.1/04 Pathogenicity by oral route

RMS comments and conclusion

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

B.6.1.2.2.1/05 Infectiveness by oral route

RMS comments and conclusion

There are no studies evaluating the infectiveness in SeMNPV. This should be considered a DATA GAP.

B.6.1.2.2.2 Acute inhalation toxicity, pathogenicity and infectiveness

Toxicological studies performed with the formulated products for other baculoviruses are considered applicable and relevant with regard to the evaluation of the active substance, since concentrations of all inert ingredients of the formulated product are without health risk.

Study results with baculoviruses are reported below.

B.6.1.2.2.2/01 Inhalation assay 01

The the characteristics and development of this study has been presented in section B.6.1.2.1/03.

Results and Conclusion

A. Results

The intradermal injection of the 12 % formulation caused slight erythema and eschar formation. No clinical signs were observed in animals of the control group. The body weight gains were not impaired. Questionable up to slight changes in the respiratory pattern were observed in the control group as well as in the test group. No significant differences were evident between the animals of both groups. Autopsy revealed no macroscopically visible abnormalities.

B. Conclusion

With the formulation Granupom no evidence for sensitisation following intradermal induction and inhalative challenge was obtained.

RMS comments and conclusion

In this study respiratory sensitisation was evaluated instead of acute inhalation toxicity. In addition, the assay was performed with Cydia pomonella GV, not the baculovirus notified.

This study is not adequate for the evaluation of acute inhalation toxicity. This should be considered a DATA GAP.

B.6.1.2.2.2/02 Inhalation assay 02

Reference	Gröner et al (1978)
Study	Investigations with baculoviruses in mammals Zeitschrift für angewandte Zoologie, 65, 69-80
Guidelines	No
Deviations	No
GLP	No
Acceptability	The study is considered additional information.

Materials and Methods

Test Substance	3 mL of an aqueous suspension containing 1×10^{10} PIB/mL of *Mamestra brassicae* NPV or 2×10^{12} granula/mL of CpGV in a 30 L sprayer.
Test animals	Guinea pigs
Method	Guinea pigs were exposed for five minutes to a virus-containing aerosol produced by fine spraying of 3 mL of an aqueous suspension, 21-day observation period. Controls received water-spray only.

Results and Conclusion

A. Results

No adverse effects on general health conditions were found as compared with the controls. Food consumption, body weight gain, body temperature and behaviour remained normal. Autopsy (including histological examinations) revealed no signs of irritation in the lungs and respiratory passages. There were no antibodies and no visible alteration in the blood electrophoretogram.

B. Conclusion

The organs of the Guinea pigs yielded no evidence of alterations due to Nucleopolyhedroviruses and Granulovirus.

RMS comments and conclusion

This study is not adequate for the evaluation of acute inhalation toxicity. This should be considered a DATA GAP.

B.6.1.2.2.2/03 Inhalation assay 03

Reference	Ignoffo et al (1975)
Study	Insusceptibility of the Rhesus Monkey, *macaca mulatta*, to an insect virus, *baculovirus heliothis* Environ Entomol, 4, 569-573 Published: yes
Guidelines	No
Deviations	No
GLP	No
Acceptability	No

Materials and Methods

Test Substance	1.2×10^8 PIB/mg Baculovirus heliothis, Heliothis zea NPV
Test animals	Rhesus monkeys 5 male & 5 female
Method	Each monkey was restrained in a headstock, the mouth was held open, and the nostrils were held close while an aerosol spray of the virus suspension (1 mg/kg, 1.2×10^8 PIB/mg) was directed to the back of the mouth. In addition two drops of the suspension were instilled in each nostril. The aerosol was produced by using a DeVilbiss nebulizer and flowmeter compressed air. The NPV was applied as single treatment and as 26 weekly treatments. Body weight gains, temperature, haematology, blood chemistry, and histopathology of treated monkeys were similar to those of untreated monkeys.

Results and Conclusion

A. Results

Body weight gains, temperature, haematology, blood chemistry, and histopathology of treated monkeys were similar to those of untreated monkeys.

B. Conclusion

The organs of the Rhesus monkeys yielded no evidence of alterations due to Heliothis zea NPV

RMS comments and conclusion

Studies carried out in primates are not accepted.

B.6.1.2.2.2/04 Other inhalation studies

Inhalation (dynamic exposure) of 3.6×10^{10} PIB/kg bw (*Orgyia pseudotsugeta* NPV) by rats, corresponding to the 60-fold field rate/ha, caused no deaths and no signs of systemic toxicity/pathogenicity. Gross pathology examinations revealed no treatment-related abnormalities (Martignoni, 1978).

Eighteen Sprague-Dawley rats were exposed for one hour to a concentrated dust of *Lymantria dispar* NPV, with a flow rate of 12 L/min. No signs of toxicity or abnormal behaviour were noted at any test animal during or following exposure. No animals died, and no treatment-related abnormalities were recorded at necropsy. Bioassay of lung, bronchii, and tracheae indicated rapid removal of NPV particles from the respiratory tract (Lewis & Podgwaite, 1981).

The inhalation of *Heliothis zea* NPV by rats did not produce any adverse effects on health (Greer, 1971, cited by Krieg, 1976).

Acute inhalation assays with *Neodiprion lecontei* NPV and with *N. sertifer* NPV on hamsters did not result in any harmful effect (Cunningham & Entwistle, 1981).

RMS comments and conclusion

The information provided is for baculoviruses that are not the virus notified by the applicant. In addition, these studies are not adequate for the evaluation of acute inhalation toxicity. This should be considered a DATA GAP.

B.6.1.2.2.2/05 Pathogenicity by inhalation

RMS comments and conclusion

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

B.6.1.2.2.2/06 Infectiveness by inhalation

RMS comments and conclusion

There are no studies evaluating the infectiveness in SeMNPV. This should be considered a DATA GAP.

B.6.1.2.2.3. Intraperitoneal/subcutaneous single dose

Toxicological studies performed with the formulated products for other baculoviruses are considered applicable and relevant with regard to the evaluation of the active substance, since concentrations of all inert ingredients of the formulated product are without health risk.

Acute i.p. injection of 1.5×10^8 and of 1.5×10^9 PIB/kg bw of *Orgyia pseudosugata* NPV into mice did not result in signs of systemic toxicity/pathogenicity, with the exception of one death at the highest dose level on the day of injection (Martignoni, 1978).

B.6.1.2.2.3/01 Intravenous tolerance assay

The the characteristics and development of this study has been presented in section B.6.1.2.2.1/01.

Results and Conclusions

A. Results

The test animals showed no changes in behaviour or adverse effects on general health condition. Body weight gains and food consumption remained unaffected by treatment with NPV.

No effect of NPV treatment on body temperature could be detected. It may be stated very confidentially that there were no changes in organ weights due to NPV.

B. Conclusion

The organs of the rats yielded no evidence of alterations due to NPV.

RMS comments and conclusion

The applicant presents an intravenous tolerance assay instead of an intraperitoneal or subcutaneous dose assay. This assay is not considered acceptable. On the other hand, the applicant has submitted other studies were intraperitoneal or subcutaneous dose tests were performed that are presented below.

B.6.1.2.2.3/02 Intraperitoneal single dose

Reference	Gröner et al (1978)
Study	Investigations with baculoviruses in mammals
Guidelines	Not stated
Deviations	Not applicable
GLP	No
Acceptability	Yes

Materials and Methods

Test Substance	1×10^9 PIB/mL of *Mamestra brassicae* NPV and 2×10^{11} granula/mL of CpGV
Test animals	Ten NMRI-mice with a starting body weight of 20-30 g
Method	Single i.p. injection of 0.5 mL of a suspension of virus inclusion bodies in physicological saline. The same amount of physiological saline was injected into mice as a control.

Results and Conclusion

A. Results

The food consumption, body weight gain and general health condition of the virus treated mice showed the same pattern as in the controls. There was no mortality or morbidity. A decline in the leucocyte values appeared one day after injection but this had returned to normal by the end of eight days (**Table 6.1.2.2.3**).

Table 6.1.2.2.3: Comparison of haematological data for mice before and after i.p. injection of nuclear polyhedra (P), together with corresponding data for untreated controls

Haemocytes	Test animals			Control animals			Normal values
	Values after injection of 0.5×10^9 P/mouse		Pre-injection (control) values	Values after injection of physiological saline		Pre-injection (control) values	Hoffmann, 1961 (cited by Gröner et al. 1978)
	day 1 p.i.	day 8 p.i.		day 1 p.i.	day 8 p i.		
Erythrocytes $\times 10^6$	10.6 ± 0.61	10.4 ± 1.46	10.9 +/ 0.93	10.2 ± 0.78	11.5 ± 0.82	10.8 ± 0.69	~ 9
Leucocytes $\times 10^3$	6.5 ± 1.40	10.5 ± 2.87	10.1 ± 5.18	9.9 ± 3.09	10 2 ± 3.92	15.6 ± 3.33	~ 10
Neutrophiles [%]	31.0 ± 8.87	32.3 ± 15.12	18.3 ± 7.85	17.6 ± 10.21	18.2 ± 13.05	16.4 ± 4.62	10…40
Eosinophiles [%]	6.3 ± 2.63	2.7 ± 3.09	2.5 ± 3.16	2.8 ± 3.42	0.2 ± 0.45	2.0 ± 1.22	0…7
Basophiles [%]	0.0	0.0	0.0	0.0	0.0	0.0	0…1
Lymphocytes [%]	62.8 ±6.50	65.0 ± 15.26	77.9 ± 10.33	79.4 ± 12.7	81.4 ± 12.60	80.8 ± 5.63	35…90
Monocytes [%]	0.0	0.0	0.46 ± 0.66	0.20 ± 0.45	0.20 ± 0.45	0.8 ± 0.84	0…3

Autopsy followed by histological examination of tissues and organs did not reveal pathological findings.

B. Conclusion

The organs of the mice yielded no evidence of alterations due to NPV and GV.

RMS comments and conclusion

Although the study has not been conducted with SeMNPV, according to the OECD Consensus Document (ENV / JM / MONO (2002) 1), baculovirus species are extremely host-specific and only occur in arthropods. Baculoviruses are not infective for mammals and replication does not occur in mammalian cells. Therefore, the study carried out with another baculovirus could be extrapolated to the one notified.

B.6.1.2.2.3/03 Subcutaneous dose assay

Reference	Ignoffo et al. (1975)
Study	Insusceptibility of the Rhesus Monkey, *macaca mulatta*, to an insect virus, *Baculovirus heliothis.*
Guidelines	Not stated
Deviations	Not applicable
GLP	No
Acceptability	No

Materials and Methods

Test Substance	1 mg/kg bw = 10^8 PIB/kg bw of *Heliothis* NPV
Test animals	Five male and six female young adult rhesus monkeys
Method	Two monkeys of each sex received a single subcutaneous dose of *Heliothis* NPV and the others received 26 weekly doses. The subcutaneous dose was administered subdermally between the scapulae with a 5-cc disposable syringe with a 20-gauge needle.

Results and Conclusion

A. Results

Body weight gains, body temperature, haematology, blood chemistry, and histopathology of treated monkeys were similar to those of untreated monkeys.

B. Conclusion

Infective virus, viral antibodies, or viral antigens were not found in blood drawn from treated animals. No additional information was identified in the literature search covering the last 10 years and focussing on toxicity or pathogenicity of baculovirus to humans and mammals.There is no evidence that SeMNPV may cause acute systemic toxicity, pathogenicity or infectivity in mammals.

RMS comments and conclusion

Studies carried out in primates are not accepted.

B.6.1.2.3. Genotoxicity testing

Since viruses are devoid of an own metabolism, biotransformation products are of no concern.

There is some evidence that vertebrate viruses themselves or their enzymes might exhibit mutagenic (mainly clastogenic) properties that can be detected by classical tests for gene mutations or chromosome aberrations (references cited by Döller and Gröner, 1983, Reimann and Miltenburger, 1983; Gröner, 1986). However, no such indications were found for baculoviruses that infect only invertebrates. The few available studies of these types on baculoviruses were all negative, in vitro as well as in vivo. Therefore no additional studies were conducted. From peer-reviewed open literature no additional reference was identified to be relevant for this data point.

B.6.1.2.3.1. In vitro studies

B.6.1.2.3.1-01 Bacterial Reverse Mutation Test

RMS comments and conclusion

The applicant has not presented a report where a bacterial reverse mutation test is carried out. In the absence of a consistent justification for the non-submission of this study, this should be considered a DATA GAP.

B.6.1.2.3.1-02 Test gene mutation in mammalian cells

RMS comment and conclusion

The applicant has not presented a report where the test for gene mutation in mammalian cells is carried out. The applicant states that virus lack of structures and mechanisms to infect mammalian cells, a test for gene mutation in mammalian cells is considered not relevant. The non-submission of a test report by the applicant is accepted since baculoviruses are entomopathogenic and need host-specific RNA to replicate. In no case would they do it in mammals.

B.6.1.2.3.1-03 Test for clastogenicity in mammalian cells

Reference	Reimann and Miltenburger (1983)
Study	Cytogenetic studies in mammalian cells after treatment with insect pathogenic viruses Baculoviridae. II In vitro studies with mammalian cell lines
Guidelines	No
Deviations	No
GLP	No
Acceptability	The study is considered additional information.

Materials and Methods

Test subtance	*Autographa californica* NPV (AcNPV) replicated in *Mamestra brassicae* cell culture (IZD-Mb-0503)
Test Item	Chinese hamster cell line B14F28 Indian muntjac cell line CCL157 Mouse cell line C3H Human lymphocytes
Test Method	Effect of baculovirus on sister chromatid exchange (SCE) rates Chromosomal aberrations

Results and Conclusion

A. Results

There was no adverse effect on cell proliferation, nor was a cytopathogenic effect (CPE) induced in such cultures. Cytogenetic data indicate that uptake of AcNPV into the cytoplasm of mammalian cells, as shown by an electron microscopic study, induced neither numerical or structural chromosome aberrations nor SCE events.

Sister Chromatid Exchanges

The Indian muntjak cells were treated for 50 h with AcNPV using a dose of 120-180 $TCID_{50}$/cell ($TCID_{50}$, tissue culture infective dose). For each experimental point at least 30 metaphases were scored. The results are summarized in **Table 6.1.2.3.1-1.** There was no difference in SCE-rates as compared with control cells ; the mean number of SCEs per chromosome was 1.5. A similar result was obtained with mouse cells which had been treated with doses of 160 to 1550 $TCID_{50}$/cell.

Table 6.1.2.3.1-1 SCEs in mammalian cell-lines after treatment with AcNPV

SCEs in 2 mammalian cell-lines after treatment with infectious supernatant from IZD-Mb-0503 cell cultures infected with Autographa californica *NPV*

Cell line	Mean chromosome number/cell	Total dose per cell ($TCID_{50}$)	Number of cells scored	Treatment hours	SCEs per chromosome
CCL 157 (Indian muntjak)	6,1	120-180	296	50 (a)	1,5
	5,9	0	326	50 (a)	1,5
C3H (mouse)	59,8	160-1550	324	50 (a)	0,16
	59,7	0	321	50 (a)	0,17

(a) 48 h culturing + 2 hours with colcemide.

Chromosomal aberrations

The results from the experiment with Chinese hamster cells and human lymphocytes are summarized in **Table 6.1.2.3.1-2.** The cultures were treated with AcNPV at a concentration of 250 $TCID_{50}$/cell. Samples of 100 metaphases with 22/23 chromosomes were scored for chromosomal aberrations. None of the samples showed increased aberration rates either at 12 or 24 h after virus inoculation.

Table 6.1.2.3.1-2 Chromosome aberrations in Chinese hamster cell line and in human lymphocytes after treatment with AcNPV

Cell type	Total dose per cell ($TCID_{50}$)	Number of cells scored	Treatment hours	Numb. of chrom. aberr. gaps	breaks	% cells with aberr. incl. gaps	excl. gaps
Chinese (a) hamster	250	100	12	1	1	2	1
	0	100	12	1	0	1	0
	250	100	24	2	0	2	0
	0	100	24	1	0	1	0
Human lymphocytes (b)	100-4000	298	72	5	3	2,7	1
	0	318	72	5	3	2,5	0,9

no exchange figures and no polyploid cells were found.

(a) only metaphases with 22/23 chromosomes were scored.
(b) only metaphases with 46 chromosomes were scored.

B. Conclusion

None of the inoculated mammalian cells showed an increase in titre or any other signs, such as delayed cell growth which might have pointed to virus replication.

The electron microscopic examinations indicated presence of AcNPV in the cytoplasm of mammalian cells but not in the nucleus and certainly not complete or partial replication.

There was no adverse effect on cell proliferation, nor was a cytopathogenic effect (CPE) induced in such cultures. Cytogenetic data indicate that uptake of AcNPV into the cytoplasm of mammalian cells, as shown by an electron microscopic study, induced neither numerical or structural chromosome aberrations nor SCE events.

RMS comments and conclusion

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

B.6.1.2.3.1-04 Other in vitro studies

In vitro investigations with AcNPV infectious supernatants (IS) in cell lines from the a.m. animals, and in primary cultures of human lymphocytes offered evidence that no increase in the chromosomal aberration and SCE rates were induced if compared with the control (Reimann, 1984).

RMS comments and conclusion

The reference Reimann 1984, is a partial translation to English of a dissertation in German, on cytogenetic effect of baculoviruses on mammalian cells in vitro and in vivo. The results included in this report are only the ones regarding the in vivo studies. There was no evidence that baculoviruses (not the baculovirus notified) had adverse effects on non-target cells. Although this information is useful it should not have been included in the in vitro studies section.

Chromosomal aberrations were not observed in vitro after exposure of vertebrate cells (frog) for four hours to nuclear polyhedrosis virions of Trichoplusia ni (McIntosh, 1975; cited by Krieg, 1976).

The applicant should have provided the article by McIntosh 1975 where chromosomal aberrations in frog cells were evaluated. The only information provided is the sentence mentioned above.

B.6.1.2.4. Cell culture study

The ability of *Heliothis zea* NPV, to infest primate cells was assessed in different human and monkey cell lines. A confluent monolayer of cells of primary African green monkey kidney, human primary embryonic kidney (HEK), human carcinoma of cervix (HeLa) and human diploid embryonic lung (W1-38) were inoculated with approx. 700 virions (*Heliothis zea* NPV)/cell. The virus did not develop in any of these cells. Cytopathic effects were not observed initially or after three serial passages through these cell types. All virus-inoculated cells failed to agglutinate guinea pig erythrocytes and presence of *H. zea* NPV did not interfere with the ability of a mammalian virus, i.e. Echo-11, to replicate in primate cells (Ignoffo & Rafajko, 1972).

The safety of *P. rapae* GV to vertebrates was documented by oral administration to numerous vertebrates and by inoculation studies: the virus was inoculated into human lung cells, rabbit kidney cells and chicken embryonic cells in tissue cultures. Neither cytopathogenic changes nor virus replication were observed under the electron microscope (Xuebao, 1982).

Seven cell lines from fish and one from an amphibian were exposed to active non-occluded OpMNPV. Cells from rainbow trout fry were also exposed to this virus by means of forced adsorption. In some experiments these cells were challenged with infectious pancreatic necrosis virus, to test for viral interference. No cytopathic effects were observed in the exposed cells, by optical and electron microscopy. No changes occurred in growth rate, nor in the cell`s response to subculture. No increase in virus titre in culture passages was demonstrable. Exposure of rainbow trout fry cells to baculovirus failed to interfere with their susceptibility to infectious pancreatic necrosis virus. In conclusion, no evidence was found that the baculovirus is capable of entering into or altering the cells used in these studies (reviewed by Martignioni, 1978).

Human HeLa cells or primary human embryonic kidney cells, simian CV1 cells, hamster BHK 21 (B3) cells or *Muntiacus muntjak* cells growing in monolayer cultures were inoculated with *Autographa californica* NPV (*Ac*NPV) at multiplicities ranging from 0.1 to 100 plaque-forming units/cell. The inoculated cells were investigated for virus production and for the replication and the persistence of viral DNA. Extracts of inoculated cells were also screened for the occurrence of AcNPV-specific RNA. Human HeLA cells in culture were inoculated with extracellular, unpurified AcNPV and the residual infectivity of the virus was measured by plaque assay at various times after inoculation. The data obtained in two independently performed experiments demonstrate that AcNPV does not replicate as infectious particles on human HeLa cells in culture. The inoculated HeLa cells did not reveal any cytopathic effects at any time after inoculation, nor was polyhedral formation observed (See **Table 6.1.2.4.1**). *Ac*NPV did not multiply in any of the cell lines studied. Viral DNA replication or transcription could not be detected by blotting and nucleic acid hybridization experiments using nick-translated, cloned viral probes. Furthermore, there was no evidence for the persistence of viral DNA or of fragments of viral DNA in mass cultures of mammalian cells (Tjia et al., 1983).

Table 6.1.2.4.1. Failure of AcNPV to replicate on human HeLa cells (Tjia et al., 1983)

FAILURE OF AcNPV TO REPLICATE ON HUMAN HeLa CELLS

Time after inoculation	Total infectivity in plaque-forming units	
	Experiment 1	Experiment 2
4 hr	1.1×10^5	7.2×10^5
5 hr	1.6×10^5	5.1×10^5
8 hr	2.4×10^5	3.8×10^5
16 hr	1.2×10^5	9.7×10^5
24 hr	0.9×10^5	8.2×10^5
Passage 2	<10	<10
Passage 4	<10	<10
Passage 17	<10	<10

At an interaction study of *Ac*NPV with the insect cell lines TN368 from *T. ni* and CP169 from *C. pomonella*, and the Chinese hamster cell line CHO-K1, all lines were able to adsorb and engulf virus particles. However, there was no evidence for viral replication in the cell lines CP169 and CHO-K1 based on virus growth titrations, electron microscopy, dot hybridisation, and synthesis of viral induced proteins (Gröner et al., 1984).

Mammalian cell cultures, i.e. a permanent cell line of human origin (Hela Ohio), one from kidney cells of monkey (Vero cells), and a primary culture of rat embryonic fibroblasts (REC) were inoculated with 50 infectious units of AcNPV per cell. Production of typical virus inclusion bodies did not occur in any cell culture tested. None of the effects characteristic of virus infection were present. Also trials failed to re-infect insect cells with culture supernatant and cell contents from the mammalian cell test. It is concluded that after three passages no virus replication had taken place (Röder & Pünter, 1977).

Activation of endogenous C-type retroviruses by polyhedra and DNA of *Ac*NPV, and by polyhedra of *M. brassicae* NPV and *L. dispar* NPV was investigated in standard cell cultures of mouse, rat, monkey and man. Cells were treated with NPV, NPV-DNA, C-type virus-activating chemicals and chemicals alone and in combination. In NPV-treated cell cultures no C-type retrovirus activation was detectable. In simultaneous treatments of the cells with NPVs and chemicals no potentiating effects by NPV could be detected. Virions of NPV in mammalian cell cultures upon re-isolation remained infectious in homologous insect cell cultures. No influence on growth or morphology of the treated mammalian cells was observed. It is concluded that the application of baculovirus for pest control is uncritical with respect to retrovirus activation in mammalian cells (Schmidt & Erfle, 1982).

The induction of antibodies might be a possible indication of baculovirus replication (Gröner, 1990). After feeding of purified *M. brassicae* NPV in the form of polyhedra (1×10^9 PIB/10 g bw) biologically active virions and UV-inactivated virions (as control) to at least 90 NMRI mice/group, no antibodies could be detected by direct solid phase radioimmunoassay (RIA) up to 60 days after infection. After aerosol application of 1×10^8 NPV/10 g bw (20 mice), also no antibody production could be observed (Döller & Gröner, 1981a).

Five pigs of ca. 18 kg bw each were force-fed 5 x 10^{10} polyhedra/kg from *M. brassicae* NPV. Five others served as controls. Although there was polyhedra-binding activity in the pig sera, this activity was non-specific: no increase in binding activity was seen during the test period of 42 days (Döller et al., 1983a). There is an unspecific interaction between CpGV and mammalian immunoglobulins. With NPV it was shown that the binding between NPV and IgG does not involve the antigen binding site of the IgG molecule but rather the Fc fragment (Döller, 1981). After oral application of GV granules to NMRI mice, virus specific antibodies could not be detected in the RIA. It is concluded that no virus replication had taken place (Döller & Huber, 1983).

The polyhedra (PH) of *M. brassicae* NPV reacted with human immunoglobulins. In the RIA, human Fa,b-fragments yielded positive reaction with PH. The antigen binding sites of anti-Hepatitis-A-virus IgG and anti-Hepatitis-A-virus Fa,b-fragments, however, were not blocked by the PH. This indicates that the interaction site is distinct from the antigen binding site. Other data indicate that at least the constant region of the heavy chain of the IgG molecule interacts with the proteins of NPV. This is an indication for an unspecific non-immunological interaction (Döller et al., 1983b).

After application of *Cydia pomonella GV* (CpGV) in a field experiment, antibodies against CpGV were detected in wild woodmice (*Apodemus sylvaticus*) but not in the bank vole (*Clethrionomys glareolus*). The seroreaction was found only in the plot treated with a tractor-drawn mist-blower and not in one treated with a hand-held spray gun. No information is possible on the type of reaction, whether it is an unspecific seroreaction or a specific antigen/antibody binding (Bailey et al., 1987).

Baculoviruses including SeMNPV are highly specific to insect cells and do not infect mammalian cells. In addition, no further information was identified in the literature search covering the last 10 years and focusing on toxicity or pathogenicity of baculovirus to humans and mammals.

RMS comments and conclusion

The applicant submits information from more than ten publications from the available literature, the most recent from 1990. None of the studies were carried out with SeMNPV but with other baculoviruses. In addition, some of the studies are not cell culture studies and should not have been included in this section (i.e. Döller & Gröner, 1981 and Döller et al., 1983a). Nevertheless, the in vivo tests performed to evaluate the replication of baculoviruses in vertebrates conclude that no virus replication had taken place in vertebrates (Döller & Gröner, 1981).
The studies reported that baculoviruses do not replicate in mammalian cells. Furthermore, there was no evidence for the persistence of viral DNA or of fragments of viral DNA in mass cultures of mammalian cells. The studies carried out with cell lines can be considered acceptable but it should be taken into account that the studies were not performed with SeMNPV.

B.6.1.2.5. Information on short term toxicity and pathogenicity

One 35-day and two 30-day acute oral tests were conducted with *Lymantria dispar* NPV products. Forty Sprague-Dawley rats were fed a single NPV dose of 2 x 10^{13} PIB/kg bw equivalent to a 100 acre dose (40 ha). Forty rats served as controls. Observations were done on mortality, behaviour, body-weight gain, food consumption, body temperature, haematology, clinical chemistry, urinanalysis, necropsy (including weight of seven organs), and histological examination of 19 tissues. No treatment related abnormalities were found (Lewis & Podgwaite, 1981).

Free but complete feeding of nutrient baits (bread) soaked in virus suspension to 20 NMRI mice/experiment (starting weight 20-30 g) was tolerated without symptoms in a single-dose study and in a repeated-dose study over 99 days. The total dose per animal was 3 x 10^{9} polyhedra (*Mamestra brassicae* NPV) or 5 x 10^{11} granula (CpGV) fed once as a single dose or distributed in 34 doses at 3 days intervals (Gröner et al., 1978). The haematological data for the treated mice (haemocyte count; differential blood count) were recorded after 3 days in the single-dose study. The haematological values for the test animals after 1 administration of viruses remained within the range of their own individual (pre-test) control values and complied to the norm (**Table 6.1.2.5-1**).

Table 6.1.2.5-1: Comparison of haematological data for mice before and after feeding a single dose of nuclear polyhedra (P) and granula (G), together with corresponding data for untreated controls (Gröner et al., 1978)

Haemocytes	Test animals		Controls		Normal values
	Values after administration of		Pre-treatment values (Control value)		Hoffmann, 1961 (cited by Gröner et al. 1978)
	3×10^9 P /mouse	5×10^{11} G /mouse			
Erythrocytes $\times 10^6$	10.0 ± 1.09	10.2 ± 1.37	10.0 ± 1.87	10.0 ± 0.78	~ 9
Leucocytes $\times 10^3$	10.1 ± 5.17	14.7 ± 5.06	13.4 ± 4.42	14.1 ± 6.45	~ 10
Neutrophiles [%]	21.1 ± 8.76	17.6 ± 7.48	18.1 ± 10.7	19.7 ± 10.1	10…40
Eosinophiles [%]	4.5 ± 2.91	2.5 ± 2.42	2.4 ± 1.92	2.5 ± 2.52	0…7
Basophiles [%]	0.0	0.0	0.0	0.0	0…1
Lymphocytes [%]	74.3 ± 9.59	79.8 ± 8.52	79.5 ± 10.4	77.6 ± 10.8	35…90
Monocytes [%]	0.19 ± 0.44	0.01 ± 0.61	0.48 ± 0.83	0.29 ± 0.46	0…3

Immediately after termination (= 100 days after the start of the study), two test animals and two controls from the repeated-dose study were selected at random for autopsy; the test animals showed no macroscopically discernible alterations compared with the untreated animals. Various organs and tissues were stained (haemalum-eosin; azan) and examined by light microscopy: digestive tract (oesophagus) stomach, ileum, colon, pancreas, liver, spleen, heart, lungs, trachea, kidney, gonads, spinal cord, and brain. Histological examination of the preparations revealed no indications of blood vessel damage (haemorrhages, exudation, and infarction), passive reactions (degeneration, atrophy or necrosis), active reactions (inflammation) or reparative or restorative processes (hypertrophy, regeneration) in the examined tissues and organs. Neoplasms were not found in either the test or control animals. No indications of autochthonic infective diseases were observed in either the test or control animals.

Feeding studies with guinea pigs (400-500 g) were conducted with a total of 5×10^{11} polyhedra (*M. brassicae* NPV) per animal (administration by intubation). This total dose was fed in 34 split doses over a period of 99 days. No adverse effects were noted. Measurements of rectal temperatures did not show signs of hyperthermia. Serum proteins (investigated with polyacrylamide gel electrophoresis) from treated animals did not differ from untreated ones. Testing for antibodies against NPV antigens in the gel diffusion test proved negative (Gröner et al., 1978).

Five male and five female young adult rhesus monkeys each received oral doses of 5 mg "*Baculovirus heliothis*" NPV/kg bw, corresponding to 6×10^8 PIB/kg bw. "*Baculovirus heliothis*" is termed *Heliothis zea* SNPV (HzSNPV) according to recent taxonomy. The doses were applied by gavage once or split-dosed weekly for 26 weeks. All monkeys were without clinical signs and showed normal gains in body weight. The average organ weights of treated animals did not differ from the untreated ones. With one exception there were no differences between the haematological values for untreated and virus-fed monkeys: one treated male had a rapid sedimentation rate (34 mm/h) and slightly elevated count (23,000/mm^3) on Day 28. Determinations taken thereafter were normal. Differences in blood chemistry between both groups were not observed. Histopathology of treated monkeys was similar to those of untreated monkeys. Infective virus, viral antibodies, or viral antigens were not found in blood drawn from treated monkeys (Ignoffo et al., 1975).

Oral short-term studies

Mice

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

Rats

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

Guinea pigs

After oral administration (by intubation) of a total of 5 x 10^{11} polyhedra (*M. brassicae* NPV) over a period of 99 days (further details see above), no differences in behaviour and body weight gain in comparison to controls were observed. Rectal temperatures were not increased. The phoretogram showed no differences to controls; in particular there were also no conspicuous alterations in the gamma-globulin fraction. Testing for antibodies against NPV antigens in the gel diffusion test proved negative (Gröner et al., 1978).

Dogs

Twenty-seven young adult purebred beagle dogs were fed *L. dispar* NPV at three dose levels (approx. 1-, 10-, and 100-acre equivalents similar to 0.4-, 4-, and 40-ha equivalents). No changes in appearance, behaviour, and appetite were observed. Treated beagle dogs showed no important changes in haematology-, clinical biochemistry- and urinalysis values. Gross pathology and histopathology examinations indicated no abnormal findings (Lewis & Podgwaite, 1981).

Monkeys

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

Inhalatory short-term studies

Rhesus monkeys

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

RMS comments and conclusion

Studies carried out in primates are not accepted.

B.6.1.2.5.1. Health effects after repeated inhalatory exposure.

Rhesus monkeys

Three male and three female young adult rhesus monkeys received 26 weekly inhalative doses of 1.2 × 10^8 PIB/kg bw of *Heliothis zea* NPV by spraying the virus suspension directly into the mouth and additional instillation of two drops in each nostril. Body weight gains, temperature, haematology, blood chemistry, and histopathology of treated monkeys were similar to those of untreated controls. Like in the oral study, a higher frequency of lymphoid hyperplasia was noted in treated monkeys than in the control animals. Infective virus, viral antibodies, or viral antigens were not found in blood drawn from treated monkeys (Ignoffo et al., 1975).

From the recent literature search, no references were identified, reporting medical cases of SeMNPV. In addition, the Occupational health statement submitted under point B.6.1.1.2. did not report any incidences related to adverse health effects.

RMS comments and conclusion

Studies carried out in primates are not accepted.

B.6.1.2.6. Proposed treatment: first aid measures medical treatment.

Likely direct or indirect adverse effects:

- May cause sensitization or an allergic reaction.

First aid measures:

- Inhalation: assure fresh air breathing. If you feel unwell, seek medical advice
- Skin contact: rinse immediately with plenty of water NO scrubbing. Take a shower for about 15 minutes. Remove contaminated shoes and clothing.
- Eye exposure: ALWAYS check for and remove contact lenses, wash eyes with plenty of water with eye lids open for at least 15 minutes
- Mouth contact or Ingestion: rinse mouth with plenty of water. Do NOT induce vomiting unless told to do so by poison control center operator or health care professional.
- If irritation or other symptoms develops, persists or worsens seek medical advice, bring packaging or label whenever possible.

NEVER LEAVE THE AFFECTED INDIVIDUAL UNATTENDED!

Advice for medical and healthcare personnel:

- Monitor vital signs and provide symptomatic and supportive treatment.
- Evaluate indication of activated charcoal.

WHEN ASKING FOR MEDICAL ADVICE KEEP PACKAGING OR LABEL AT HAND AND CALL YOUR LOCAL POISON CONTROL CENTER ([*INSERT LOCAL NUMBER HERE*].

B.6.2. TIER II

B.6.2.1. Specific toxicity, pathogenicity and infectiveness studies

General remark: The studies reported under **B.6.1.1** up to **B.6.1.2** show that baculoviruses do not produce toxic effects to animals and man. Further conduction of specific toxicity, pathogenicity and infectiveness studies, therefore, is of no obligation. Toxicological studies performed with the formulated products for other baculoviruses are considered applicable and relevant with regard to the evaluation of the active substance, since concentrations of all inert ingredients of the formulated product are without health risk.

Carcinogenicity: *Heliothis zea* NPV polyhedra were injected subcutaneously into neonate rats and intravenously into adult rats. Rats were also fed in a 2-year study with 6×10^9 PIB/100 g diet. There was no significant change in the rate of spontaneous tumour formation in any of those tests (reviewed by Krieg, 1976).

Lymantria dispar NPV was fed to albino rats over a period of 2 years. Total ingestion of PIBs equaled 10-fold and 100-fold the field equivalent dose. One hundred rats were used per test dose plus 100 rats as controls. Treatment did not influence survival, body weight, or food consumption. The tumour incidence or other microscopic lesions found were not attributable to the treatment (reviewed by Lewis & Podgwaite, 1981).

Teratogenicity: Twenty-four female mated rats were fed with *Heliothis zea* NPV diseased larval tissue, containing 1×10^9 PIB/kg bw/day, during the 5^{th} to 14^{th} day of gestation. Twenty-five dams receiving healthy larval tissue served as controls (Ignoffo et al., 1973). None of the virus-fed or control rats showed abnormalities in general physical condition, behaviour, or in body weight gain. Administration of NPV to pregnant dams did not affect number or weight of fetuses, resorption sites, or relationship between corpora lutea and implantation site (**Table.6.2.1-1**).

Table.6.2.1-1: Summary of results from examinations of ovaries, uteri, and fetuses from dams fed virus or healthy tissue during the 5th to 14th day of gestation

Average no./pregnant female[a]						
Treatment[b]	**No. females**	**Resorption sites**	**Implantation sites**	**Corpora lutea**	**Viable fetuses**	**Fetal weight (g)**
Virus	24	0.8 ± 0.2	8.0 ± 0.7	9.0 ± 0.8	7.3 ± 0.6	3.5 ± 0.2
Control	25	0.6 ± 0.2	6.6 ± 0.8	7.4 ± 1.0	6.1 ± 0.8	3.5 ± 0.1

[a] Average ± standard error of mean
[b] Virus dose 1 x 10^9 PIB/kg bw per day

No teratological abnormalities were found in 20-day old rat fetuses. Visceral and skeletal abnormalities when present were randomly distributed throughout both virus-fed and non-virus-fed dams, and were within expected limits for 20-day old fetal rats. None of the changes could be attributed to NPV application (Ignoffo et al., 1973).

A dose of 0.2 mg virions from CpGV were administered orally to five gravid NMRI mice and to five *in oestrus* prior to mating. The 78 pups from these litters did not differ from controls with regard to growth and development. Serum was obtained four weeks after the birth of pups and tested in the RIA. Virus-induced antibodies could not be detected in any of the sera (Döller & Huber, 1983).

No abnormalities were observed in one pregnant rhesus monkey nor in her male offspring after she had received three subcutaneous injections each of 1 mg *Heliothis zea* NPV/kg bw, containing 1.2 x 10^8 PIB, before giving birth (Ignoffo et al., 1975).

From the recent literature search, no relevant references were identified (please see section B.6.1.1).

RMS comments and conclusion

According to literature research, baculoviruses do not cause long-term health effects, so studies on chronic toxicity, pathogenicity and infectiveness, carcinogenicity and reproductive toxicity could be waived. Nevertheless, the applicant presents information of reports where carcinogenicity and teratogenicity of baculoviruses were assessed and no teratogenic or carcinogenic effects were observed for the baculoviruses tested. The information provided is considered acceptable.

B.6.2.2. In vivo studies in somatic cells

In vivo experiments were conducted with CpGV granula to the Chinese hamster. A single dose of 1.5 x 10^{12} granula/animal and daily doses of 1.6 x 10^{10} granula/animal over a 90-d period were orally administered by intubation. The test animals were prepared 24 hours after the final virus dose. The sister chromatid exchange (SCE) in bone marrow cells from single-dose animals as well as the number of chromosomal aberrations in bone marrow cells from both dosage groups were determined and compared to those from the controls. No changes could be detected. Corresponding *in vivo* trials with *M. brassicae* NPV-PIBs and *Ac*NPV-infections supernatant (IS) gave similar results (Reimann, 1984).

The chromosome aberration rate was determined in bone marrow smears from 24 mice after single and repeated-dose feeding studies with granula (CpGV) and nuclear polyhedra (*M. brassicae* NPV) and also from four guinea pigs after a repeated-dose feeding study with nuclear polyhedra (doses and frequencies of feeding see under point B.6.2.1) There was no evidence of an increase in the aberration rate among test animals as compared with controls (Gröner et al., 1978).

No indications of genotoxicity are known for SeMNPV (please refer to Point B.6.1.2.3. above). Therefore, studies on genotoxic effects in somatic cells were not considered necessary.

RMS comments and conclusion

The reports are considered acceptable although they were not performed with SeMNPV.

B.6.2.3. Genotoxicity- *In vivo* studies in germ cells

Since all *in vitro* and *in vivo* studies with baculoviruses in somatic cells were negative, no *in vivo* studies in germ cells are required.

RMS comments and conclusion

The non-submission of information is considered acceptable

B.6.3. SUMMARY OF MAMMALIAN TOXICITY, PATHOGENICITY AND INFECTIVENESS AND OVERALL EVALUATION OF THE ACTIVE MICRO-ORGANISM

Table.6.3 Overview of the available data

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

Baculoviruses have a narrow host range and are strictly host-specific to certain arthropod species (OECD, 2002,). Baculoviruses are part of our natural environment and there is no evidence that they may have caused any disease process in humans or other mammals. In fact, these viruses do not replicate in vertebrate cells. According to the whole available knowledge, the *Spodoptera exigua* multicapsid nucleopolyhedrovirus (SeMNPV) does not affect any organism except lepidopteran insects of the species *Spodoptera exigua*. Toxic metabolites or degradation products do not occur. For a virus, potential formation of substance with antimicrobial activity or the development and spread of resistance to antibiotics of medical importance is not of concern.

No adverse effects including allergic reactions (neither by skin contact nor by inhalation) have been reported in humans who were in close contact with baculoviruses.

Basic studies with baculoviruses or its formulations in laboratory animals using the oral, inhalative or intraperitoneal routes were carried out confirming that this virus is not infectious, pathogenic or toxic to mammals. Repeated oral administration to mice was tolerated without any adverse effects.

SeMNPV is devoid of a genotoxic (clastogenic) potential and did not produce effects on a molecular level in a cell culture study. In fact, it was shown that some baculoviruses under certain conditions may enter cells of human origin but fails to replicate there and to damage the host cells.

The favourable risk assessment of SeMNPV is further supported by extensive information that was obtained with other baculoviruses. This data covers further endpoints and is considered applicable to SeMNPV. Although of different quality and reliability, these studies and publications, in the whole, did not elucidate a health risk to mammals. All baculoviruses tested so far proved non-pathogenic when administered to various mammalian species in single dose experiments by various routes of exposure. Likewise, repeated exposure in short- and long-term studies did not result in adverse health effects, however, most studies of these types were of limited scientific value because of deficiencies with regard to conduct and reporting. There is no evidence of mutagenicity and cell culture studies demonstrated the lack of infectivity to vertebrate cells as well as the absence of interaction with cellular mammalian DNA. Activation of "silent" genes of retroviral origin was not observed. Baculoviruses are apparently devoid of a cancerogenic or teratogenic potential and do not affect fertility or reproduction.

Some seroconversion (antibody formation) may occur in different species including man but this is considered a rather unspecific immunological response and not indicative of a productive infection with virus replication.

Baculoviruses such as SeMNPV may be generally considered as safe with regard to human and animal health. Of course, standard hygienic procedures should be maintained when handling virus formulations.

As for other micro-organisms and viruses, it is not possible and not necessary to derive reference doses.

In summary, no toxicity or infectivity was noted in experimental studies upon oral, dermal, respiratory or intraperitoneal exposure even to exceedingly high dose levels (**Table 6.3**). Taking together the results of these experimental studies, of epidemiological and occupational evidence, and the experience from several decades of safe application of CpGV-based plant protection products it is appropriate to state that there is no concern with regard to human health.

RMS comments and conclusion

Numerous studies were carried out; however, none were conducted with SeMNPV. According to the OECD Consensus Document (ENV / JM / MONO (2002) 1), baculovirus species are extremely host-specific and only occur in arthropods. Baculoviruses are not infective for mammals and replication does not occur in mammalian cells. Therefore, studies carried out with another baculovirus could be extrapolated to the one notified.

Although the applicant states that the virus is neither infectious nor pathogenic nor toxic, the opinion of the RMS is that this is a DATA GAP, since it has not been evaluated for the notified baculovirus and the studies do not assess ineffectiveness.

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

In summary, there is lack of information about the toxicity, pathogenicity and ineffectiveness of the virus. In addition, according to Regulation (EC) 283/2013, all microorganisms should be regarded as potencial sensitisers.

B.6.4 REFERENCES RELIED ON (ABA, ANDERMATT BIOCONTROL AG)

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
B.6.1.1/01	OECD	2002	Consensus document on information used in the assessment of environmental applications involving baculovirus ENV/JM/MONO, 1, 1-90 No GLP Published	N	N		-
B.6.1.1/02	Jehle, J.A., et al.	2006	Molecular identification and phylogenetic analysis of baculoviruses from Lepidoptera Virology, 346, 180-193 No GLP Published	N	N		-
B.6.1.1/03	Rohrmann, G.F.	2013	Chapter 1: Introduction to the baculoviruses, their taxonomy and evolution Baculovirus Molecular Biology, 3rd edition, 1-24 No GLP Published	N	N		-
B.6.1.1/04	Lung, O.	2002	Pseudotyping *Autographa californica* Multicapsid Nucleopolyhedrovirus (AcMNPV): F Proteins from Group II NPVs Are Functionally Analogous to AcMNPV GP64 Virology, 346, 180-193 No GLP Published	N	N		-
B.6.1.1/05	Krieg, A.	1976	Granulosis and nuclear polyhedrosis viruses: Safety aspects concerning their production and application Z Angew Entomol, 82, 129-134 No GLP Published	N	N		-
B.6.1.1/06	Seehase, S.	2018	Literature review on *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) toxicology Andermatt Biocontrol AG, CH, 159365-MA-05-01 No GLP Unpublished	N	Y	Propietary information	ABA
B.6.1.1.1/01	Gronowski, A.M., et al	1999	Baculovirus stimulates antiviral effects in mammalian cells Journal of Virology, 73, 9944-9951 No GLP Published	N	N		-

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
B.6.1.1.1/02	Ignoffo, C.M.	1973	Effects of entomopathogens on vertebrates Annals of the New York Academy of Sciences, 217, 141-172 No GLP Published	Y	N		-
B.6.1.1.1/03	Burges, H.D.; et al	1980	A review of safety tests on baculoviruses Entomophaga, 25 (4), 329-339 No GLP Published	N	N		-
B.6.1.1.1/04	Gröner, A.	1986	Specificity and safety of baculoviruses The Biology of Baculoviruses, Volume I, Biological Properties and Molecular Biologie, Chapter 9, 177-201 No GLP Published	N	N		-
B.6.1.1.2	Zingg, D.	2018	Occupational health statement Andermatt Biocontrol AG, CH, No GLP Unpublished	N	Y	Propietary information	ABA
B.6.1.1.3/01	Andermatt, M.	2006a	Declaration Andermatt Biocontrol AG, CH, Andermatt Biocontrol AG, Grossdietwil, Switzerland No GLP Unpublished	N	N		ABA
B.6.1.1.3/02	Andermatt, M.	2006b	Statement on the production of baculovirus products of Andermatt Biocontrol AG and on its workers exposure taking account of potential risks of inhalation toxicity Andermatt Biocontrol AG, Grossdietwil, Switzerland No GLP Unpublished	N	N		ABA
B.6.1.1.3/03	Zingg, D.	2018	Occupational health statement Andermatt Biocontrol AG, CH, No GLP Unpublished	N	Y	Propietary information	ABA
B.6.1.1.3/04	Seehase, S.	2018	Literature review on *Spodoptera exigua* multiple nucleopolyhedrovirus (SEMNPV) toxicology Andermatt Biocontrol AG, CH, 159365-MA-05-01 No GLP Unpublished	N	Y	Propietary information	ABA

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
B.6.1.1.4	Heimpel, A.M., Buchanan, L.K.	1967	Human feeding tests using a nuclear-polyhedrosis virus of *Heliothis Zea* Journal of Invertebrate Pathology, 9, 55-57 No GLP Published	N	N		-
B.6.1.2.1/01	[redacted]	1976a	Testing for senitising properties of nuclear polyhedrosis virus (NPV) in guinea pigs by the method of LANDSTEINER Andermatt Biocontrol GmbH, Report No.148/76, A55527 [redacted] No GLP Unpublished	Y	N		ABA
B.6.1.2.1/02	[redacted]	1986	HOE 083311 OI LC08 A101 Testing for sensitising properties of on Pirbright White guinea pigs by the method of LANDSTEINER Andermatt Biocontrol GmbH, 861169, 86.1373 [redacted] GLP Unpublished	Y	N		ABA
B.6.1.2.1/03	[redacted]	1992	Hoe 083311; Water miscible suspension concentrate: 2.2*10 exp. 13 vir./1 (code: Hoe 083311 00 SC13 A102) Testing for respiratory sensitization in the male and female Pirbright White guinea pig. Report No. 91.1096 Andermatt Biocontrol GmbH, [redacted] GLP Unpublished	Y	N		ABA
B.6.1.2.1/04	Hackl et al	2015	Literature search and data collection on risk assessment for human health for microorganisms used as plant protection products reference. EFSA supporting publication 2015:en-801. 173 pp				
B.6.1.2.1/05	Martel et al.	(2010)	Bibliographic review on the potential of microorganisms, microbial products and enzymes to induce respiratory sensitization. EFSA supporting publication 2010 volume 7, issue 9, 95pp				
B.6.1.2.2.1/01	[redacted] et al.	1976b	Tolerance testing with nuclear polyhedrosis virus after single	Y	N		ABA

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
			oral or intravenous administration to male and female rats Report No. 488/76 Andermatt Biocontrol GmbH, [redacted] No GLP Unpublished				
B.6.1.2.2.1/02	[redacted]	1980	Tolerance testing of (AcNPV) nuclear polyhedrosis virus following single-dose administration to SPF wistar rats Andermatt Biocontrol GmbH, 595, 234/80 [redacted] GLP Unpublished	Y	N		ABA
B.6.1.2.2.1/03	Martignoni, M.E.	1978	The Douglas-fir tussock moth: a synthesis Forest Ser. Tech. Bulletin 1585. U.S. Dep. of Agriculture, ed. by: Brookes, M.H., Stark, R.W., Campell, R.W. No GLP Published	N	N		-
B.6.1.2.2.1/04	Cunningham, J.C., Entwistle, P.F.	1981	Control of sawflies by baculovirus. IV Characterization and safety testing. Microbial control of pests and plant diseases, 392-393 No GLP Published	N	N		-
B.6.1.2.2.1/05	Xuebao, W.	1982	Safety tests of a GV insecticide against cabbage butterfly *Pieris rapae* larvae RAE Serie A, 70 (4), 2368 No GLP Published	N	N		-
B.6.1.2.2.2/01	[redacted]	1992	Hoe 083311; water miscible suspension concentrate: 2.2*10 exp. 13 vir./1 (code: Hoe 083311 00 SC13 A102) Testing for respiratory sensitization in the male and female Pirbright White guinea pig. Report No. 91.1096 Andermatt Biocontrol GmbH, [redacted] GLP Unpublished	Y	N		ABA

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
B.6.1.2.2.2/02	Gröner, Aet al	1978	Investigations with baculoviruses in mammals Zeitschrift für angewandte Zoologie, 65, 69-80 No GLP Published	Y	N		-
B.6.1.2.2.2/03	Ignoffo, C.M., et al .	1975	Insusceptibility of the Rhesus Monkey, *Macaca mulatta*, to an insect virus, *Baculovirus heliothis* Environ Entomol, 4, 569-573 No GLP Published	Y	N		-
B.6.1.2.2.2/04	Martignoni, M.E.	1978	The Douglas-fir tussock moth: a synthesis Forest Ser. Tech. Bulletin 1585. U.S. Dep. of Agriculture, ed. by: Brookes, M.H., Stark, R.W., Campell, R.W. No GLP Published	N	N		
B.6.1.2.2.2/05	Lewis, F.B., Podgwaite, J.D.	1981	The gypsy moth: research toward integrated pest management - safety evaluations Technical Bulletin, U.S. Department of Agricultur, 1584, 475-479 No GLP Published	N	N		-
B.6.1.2.2.2/06	Krieg, A.	1976	Granulosis and nuclear polyhedrosis viruses: safety aspects concerning their production and application Z Angew Entomol, 82, 129-134 No GLP Published	N	N		-
B.6.1.2.2.2/07	Cunningham, J.C., Entwistle, P.F.	1981	Control of sawflies by baculovirus. iv characterization and safety testing. Microbial control of pests and plant diseases, 392-393 No GLP Published	N	N		-
B.6.1.2.2.3/01	[redacted] et al.	1976b	Tolerance testing with nuclear polyhedrosis virus after single oral or intravenous administration to male and female rats Report No. 488/76 Andermatt Biocontrol GmbH, [redacted] No GLP Unpublished	Y	N		ABA

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
B.6.1.2.2.3/02	Gröner, A., et. al.	1978	Investigations with baculoviruses in mammals Zeitschrift für angewandte Zoologie, 65, 69-80 No GLP Published	Y	N		-
B.6.1.2.2.3/03	Ignoffo, C.M.et al.	1975	Insusceptibility of the Rhesus Monkey, *Macaca mulatta*, to an insect virus, Baculovirus *heliothis* Environ Entomol, 4, 569-573 No GLP Published	Y	N		-
B.6.1.2.3.1/01	Reimann, R., Miltenburger, H.G.	1983	Cytogenetic studies in mammalian cells after treatment with insect pathogenic viruses [baculoviridae]. II In vitro studies with mammalian cell lines Entomophaga, IOBC journal, 28, 33-44 No GLP Published	N	N		-
B.6.1.2.3.1/02	Gröner, A.	1986	Specificity and safety of baculoviruses The Biology of Baculoviruses, Volume I, Biological Properties and Molecular Biologie, Chapter 9, 177-201 No GLP Published	N	N		-
B.6.1.2.3.1/03	Reimann, R.K.H.	1984	Cytogenetic investigations of the effect of viral insect pathogens (baculoviruses) on mammalian cells in vivo and in vitro (german original) Dissertation Technische Hochschule Darmstadt No GLP Published	Y	N		-
B.6.1.2.3.1/04	Krieg, A.	1976	Granulosis and nuclear polyhedrosis viruses: safety aspects concerning ther production an application [german original] Zeitschrift für angewandte Entomologie, 82, 129-134 No GLP Published	N	N		-
B.6.1.2.4/01	Ignoffo, C.M., Rafajko, R.R.	1972	In vitro attempts to infect primate cells with the nucleopolydrosis virus of *heliothis* Journal of Invertebrate Pathology, 20, 321-325 No GLP Published	N	N		-

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
B.6.1.2.4/02	Xuebao, W.	1982	Safety tests of a GV insecticide against cabbage butterfly *Pieris rapae* larvae RAE Serie A, 70 (4), 2368 No GLP Published	N	N		-
B.6.1.2.4/03	Martignoni, M.E.	1978	The Douglas-fir tussock moth: a synthesis Forest Ser. Tech. Bulletin 1585. U.S. Dep. of Agriculture, ed. by: Brookes, M.H., Stark, R.W., Campell, R.W. No GLP Published	N	N		-
B.6.1.2.4/04	Tjia, S., et al.	1983	*Autographa californica* nuclear polyhedrosis virus (ACNPV) DNA does not persist in mass cultures of mammalian cells Virology 125, pp 107-117 No GLP Published	N	N		-
B.6.1.2.4/05	Gröner, A., et al.	1984	Interaction of *Autographa californica* nuclear polyhedrosis virus with two nonpermissive cell lines Intervirology 21: pp. 203-209 (1984) No GLP Published	N	N		-
B.6.1.2.4/06	Röder, A., Pünter, J.	1977	Interactions between nuclear polyhedrosis viruses and vertebrate cells Zentralblatt für Baktgeriologie und Hygiene I Abteilung Original, A 239, 459-464 No GLP Published	N	N		-
B.6.1.2.4/07	Schmidt, J., Erfle, V.	1982	Studies on the retrovirus-activating potential of nuclear polyhedrosis viruses in mammalian cell cultures Zbl. Bakt. Hyg., I. Abt. Orig. A 252, pp. 438-455 GLP/GEP: no Published: yes	N	N		-
B.6.1.2.4/08	Gröner, A.	1990	*Cydia pomonella granulosis* virus (CPGV) HOE 083311 summary and conclusions on the toxicity Andermatt Biocontrol GmbH, AgrEvo, Hoechst and Schering, Marburg, Germany No GLP Unpublished	N	N		ABA
B.6.1.2.4/9	Döller, G., Gröner, A.	1981a	Safety test for the control of virus replication of nuclear	N	N		-

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
			polyhedrosis virus from *Mamestra brassicae* in vertebrates (german orignal) Z Angew Entomol, 92, 99-105 No GLP Published				
B.6.1.2.4/10	Döller, G.et al	1983a	Safety evaluation of nuclear polyhedrosis virus replication in pigs Applied and Environmental Microbiology 45 (4): pp. 1229-1233 No GLP Published	N	N		-
B.6.1.2.4/11	Döller, G.	1981b	Unspecific interaction between granulosis virus and mammalian immunoglobulins Naturwissenschaften, 68, 1-2 No GLP Published	N	N		-
B.6.1.2.4/12	Döller, G., Huber, J.	1983b	Safety test for the control of virus replication of granulosis virus form *Laspeyresia pomonella* in mammalians (german original) Z Angew Entomol, 95, 64-69 No GLP Published	N	N		-
B.6.1.2.4/13	Bailey, M.J.et al	1987	Specific immunological response against the granulosis virus of the codling moth (*Cydia pomonella*) in woodmice (*Apodemus sylvaticus*): field observations Annals of Applied Biology, 111, 649-660 No GLP Published	N	N		-
B.6.1.2.5/01	Lewis, F.B., Podgwaite, J.D.	1981	The gypsy moth: research toward integrated pest management - safety evaluations Technical Bulletin, U.S. Department of Agricultur, 1584, 475-479 No GLP Published	N	N		-
B.6.1.2.5/02	Gröner, A.et al	1978	Investigations with baculoviruses in mammals Zeitschrift für angewandte Zoologie, 65, 69-80 No GLP Published	Y	N		-
B.6.1.2.5/03	Ignoffo, C.M.et al .	1975	Insusceptibility of the Rhesus Monkey, *Macaca mulatta*, to an insect virus, Baculovirus *heliothis* Environ Entomol, 4, 569-573 No GLP	Y	N		-

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
			Published				
B.6.1.2.5/04	Cunningham, J.C., Entwistle, P.F.	1981	Control of sawflies by baculovirus. iv characterization and safety testing. Microbial control of pests and plant diseases, 392-393 No GLP Published	N	N		-
B.6.1.2.5.1	Ignoffo, C.M.et al	1975	Insusceptibility of the Rhesus Monkey, *Macaca mulatta*, to an insect virus, Baculovirus *heliothis* Environ Entomol, 4, 569-573 No GLP Published	Y	N		-
B.6.2.1/01	Krieg, A.	1976a	Granulosis and nuclear polyhedrosis viruses: safety aspects concerning their production and application Z Angew Entomol, 82, 129-134 No GLP Published	N	N		-
B.6.2.1/02	Lewis, F.B., Podgwaite, J.D.	1981	The gypsy moth: research toward integrated pest management - safety evaluations Technical Bulletin, U.S. Department of Agricultur, 1584, 475-479 No GLP Published	N	N		-
B.6.2.1/03	Ignoffo, C.M.et al	1973	Teratogenic potential in rats fed the nuclear polyhedrosis virus of *heliothis* Environ Entomol, 2, 337-338 No GLP Published	Y	N		-
B.6.2.1/04	Döller, G., Huber, J.	1983	Safety test for the control of virus replication of granulosis virus form *Laspeyresia pomonella* in mammalians (german original) Z Angew Entomol, 95, 64-69 No GLP Published	Y	N		-
B.6.2.1/05	Ignoffo, C.M.et al	1975	Insusceptibility of the Rhesus Monkey, *Macaca mulatta*, to an insect virus, Baculovirus *heliothis* Environ Entomol, 4, 569-573 No GLP Published	Y	N		-
B.6.2.2/01	Reimann, R.K.H.	1984	Cytogenetic investigations of the effect of viral insect pathogens (baculoviruses) on mammalian cells in vivo and in vitro (german original) Dissertation Technische Hochschule Darmstadt	N	N		-

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
			No GLP Published				
B.6.2.2/02	Gröner, A.et al	1978	Investigations with baculoviruses in mammals Zeitschrift für angewandte Zoologie, 65, 69-80 No GLP Published	Y	N		-