Renewal Assessment Report

Cydia pomonella GV

Volume 3 – B.5 Analytical methods

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The RMS is the author of the Assessment Report. The Assessment Report is based on the validation by the RMS, and the verification during the EFSA peer-review process, of the information submitted by the Applicant in the dossier, including the Applicant's assessments provided in the summary dossier. As a consequence, data and information including assessments and conclusions, validated and verified by the RMS experts, may be taken from the applicant's (summary) dossier and included as such or adapted/modified by the RMS in the Assessment Report. For reasons of efficiency, the Assessment Report should include the information validated/verified by the RMS, without detailing which elements have been taken or modified from the Applicant's assessment. As the Applicant's summary dossier is published, the experts, interested parties, and the public may compare both documents for getting details on which elements of the Applicant's dossier have been validated/verified and which ones have been modified by the RMS.

Table of contents

B Summary of the data and information

B.5 Analytical methods 4
B.5.1 Methods for the analysis of the micro-organism as manufactured 4
B.5.1.1 Methods for the identification of the micro-organism 4
B.5.1.2 Methods for providing information on possible variability of seed stock/active micro-organism 8
B.5.1.3 Methods to differentiate a mutant of the micro-organism from the parent wild strain 9
B.5.1.4 Methods for the establishment of purity of seed stock from which batches are produced and methods to control that purity 9
B.5.1.5 Methods to determine the content of the micro-organism in the manufactured material used for the production of formulated products and methods to show that contaminating micro-organisms are controlled to an acceptable level 9
B.5.1.6 Methods for the determination of relevant impurities in the manufactured material 11
B.5.1.7 Methods to control the absence and to quantify (with appropriate limits of determination) the possible presence of any human and mammalian pathogen 11
B.5.1.8 Methods to determine storage stability, shelf-life of the micro-organism, if appropriate 12
B.5.2 Methods to determine and quantify residues (viable or non-viable) of the active micro-organism 12
B.5.2.1 The active micro-organism(s) on and/or in crop, in foodstuffs and feeding stuffs, in animal and human body tissues and fluids, in soil, in water (including drinking water, ground water and surface water) and in air where relevant (i.e. viable residues) 12
B.5.2.2 Relevant metabolites (especially toxins) on and/or in crop, in foodstuffs and feeding stuffs, in animal and human body tissues and fluids, in soil, in water (including drinking water, ground water and surface water) and in air where relevant (i.e. non -viable residues) 13
B.5.3 References relied on 14

B.5 Analytical methods

B.5.1 Methods for the analysis of the micro-organism as manufactured

In the DAR only CPGV Mexican isolate was evaluated. Several additional isolates were evaluated according to the "Guidance Document SANCO/0253/2008 on the assessment of new isolates of baculovirus species already included in Annex I of Council Directive 91/414" A Report was written for each isolate and the MS were given the opportunity to comment. After taking note at the SCFCAH the respective isolate was added to Appendix III of the Review Report.
Studies submitted for the additional isolates are considered as new information for the renewal.

Serbios srl does not produce its own MPCA. Therefore, no data for MPCA are provided in this chapter for Serbios srl.

B.5.1.1 Methods for the identification of the micro-organism

Andermatt Biocontrol GmbH

Information already presented in the DAR

Reference:
Harvey, J.P. and Volkman, L.E., 1983, Biochemical and biological variation *of Cydia pomonella* (codling moth) Granulosis virus (BWS 2003-103)

The identity of the virus produce can be bioanalytically checked against the parent strain by
- SDS-polyacrylamide-gel electrophoresis of the virus proteins
- Restriction endonuclease analysis of viral DNA

Methods:
SDS-polyacrylamide-gel electrophoresis of the virus proteins:
Viral proteins are electrophoresed in 10 and 12 % polyacrylamide gels. The proteins are denatured by boiling for 3 min in a disruption buffer of 0.25 M Tris (pH 6.8), 5 % mercaptoethanol, 1 % SDS, and 10 % glycerol. They are loaded immediately into the sample wells of the gel, and electrophoresed for 3 h at a constant current of 30 mA/gel. After electrophoresis, the resolving gels are routinely fixed in 25 % isopropanol, 10 % acetic acid, then stained for 4 h with 0.04 % Coomassie blue R250, and destained overnight in 7 % acetic acid. Molecular weight standards from Bethesda Research Laboratories (myosin, MW 2,000,000; phosphorylase b, MW 92,500; bovine serum albumin, MW 68,000; ovalbumin, MW 45,000; *a*-Chymotrypsinogen, MW 25,700; *ß*-lactoglobulin, MW 18,400; cytochrome c, MW 12,300) are electrophoresed on gels alongside the samples. Standard curves relating relative mobility of the standards with the logarithm of molecular weights are drawn, and using the method of WEBER and OSBORN (1969) cited by Harvey and Volkman (1983) the molecular weights of the CpGV viral proteins are determined.

Restriction endonuclease analysis of viral DNA:
DNA *purification*: Gradient purified enveloped virions at a concentration of 5 mg/mL are heated to 65 °C for 15 min to destroy nucleases prior to a 30-min incubation in 1 % sodium dodecyl sulfate (SDS) at 65 °C. Proteinase K (Sigma Chemical Co., St. Louis, Mo.) is added to each sample to a concentration of 100 µg/mL and incubated for 1 h at 37 °C. The DNA is extracted with phenol, equal volumes phenol and chloroform:isoamyl alcohol (24:1), and then by chloroform:isoamyl alcohol (24:1) alone. The purified DNA is dialysed extensively against three changes of 0.1 x SSC buffer (0.15 *M* sodium chloride, 0.015 *M* sodium citrate) for a 24-h period at 4 °C, and then stored in sterile minifuge tubes at 4 °C. The concentration is determined from the OD_{260} using an absorbance of 20 for

1 mg/mL concentration. All DNA used had an OD_{260}:OD_{280} ratio of 1.8+/-0.05, indicating relatively pure preparations.

Restriction endonuclease analysis: The DNA is digested for 2-4 h at 37 °C with 5 units of restriction enzyme *Bam*HI, *Hind*III, *Eco*RI, *Xho*I, *Sal*I, or *Kpn*I from Bethesda Research Laboratories (BRL, Rockville, Md.) using the specific assay buffers suggested by the supplier. In case of partial digestions, another 5 units of enzyme are added per sample and the reaction mixture reincubated for 4 h. The digested DNA samples are fractionated on 0.7 % agarose gels in a BRL horizontal gel unit at 60 V for 14 h, stained with ethidium bromide, and visualised under short wave UV light. Molecular weight estimates of CpGV fragments are determined from a plot of molecular weights versus migration distance using lambda DNA digested with *Hind*III and AcMNPV DNA digested with *Eco*RI as standards. The gels are photographed using Kodak Plus-X film, illumination from an ultra-violet light box, and a Wratten No. 15 filter.

Conclusion by RMS
The study is still considered acceptable.

New information for RAR
The morphology of CpGV can be studied under the electron microscope in ultrafine sections. The dimensions of the nucleocapsid are 180-200 x 390 nm. The inclusion bodies have an ovoid-cylindrical form with length 200-230 nm and diameter 34-36 nm. Different species of granuloviruses cannot be distinguished by microscopic techniques.

A first molecular analysis of the CpGV genome was done by Crook et al. (1997). The entire genome sequence of CpGV is available at GenBank under the number U53466 (NCBI Sequence Viewer v2.0, 2001). The nucleotide sequence of the DNA of the CpGV is made up of 123500 base pairs (Luque et al., 2001). Circa 143 putative genes have been identified, of which 73 are similar to genes of the *Autographa californica* Nucleopolyhedrovirus (AcMNPV), 108 are similar to genes of Xestia c-nigrum GV (XecnGV), and 98 are similar to genes from *Plutella xylostella* GV (PlxyGV). The similarity to genes from XecnGVand PlxyGV confirms the belonging of CpGV to the group of granuloviruses.

Three different CpGV isolates are reported in the literature. The first isolate was found in Mexico in 1963 and named Mexican isolate (CpGV-M). Later, the English (CpGV-E) and Russian (CpGV-R) isolates were discovered. The Mexican isolate is used in Madex and other commercially available products. Baculovirus isolates are not genetically uniform and always represent a certain degree of heterogeneity, which can be seen in restriction patterns. This heterogeneity is due to the presence of deletions or insertions within the viral genomes.

Additional isolates from Andermatt Biocontrol GmbH that were included in Annex III of the Review Report for CpGV are: CpGV-V15 (GV-0013), CpGV-V22 (GV-0014), CpGV-V03 (GV-0006, Madex Max) and CpGV-V01 (GV-0003, Madex Plus). Methods for identification of these isolates are described in Vol. 3 MA B.1 and Vol. 4 for Andermatt Biocontrol GmbH.

Arysta LifeScience S.A.S

Information already presented in the DAR
The Baculoviruses can be identified by means of electrophoretic profiles obtained by separation of DNA genome fragments generated by the restriction enzymes.

- This technique, used currently in virological laboratories consists of:
- purification of the granules by centrifugation of virosed dead larvae;
- purification of the virions contained in the granules after solubilisation of these latter in an alkaline environment. The virions are isolated on sucrose gradient;

- extraction of DNA using phenol, after digestion of virion proteins by proteinases;
- fractionating of the DNA molecules by the use of restriction endonucleases;
- separation of the fragments obtained by electrophoresis.

This technique is efficient and allows:
- examination of the entire genome and not only the structural genes,
- discrimination between closely related strains,
- detection of a mixture of viral strains,
- detection of eventual insertions or deletions of DNA in an already described strain.

Reference:
Croizier, G., 2001. Analyse des virus de la granulose de *Carpocapsa pomonella.* Standard NPP 1996 et Standard NPP 2001. INRA (BWS 2006-83)

The material, consisted of two aqueous suspensions of purified granules. The quantity of standard NPP 1996 was limited enabling only one DNA direct extraction after alkaline solubilisation of granules and excluding a preliminary purification of viral particles. The quantity of standard NPP 2001 was largely sufficient. A large sample of non purified material dating from before 1991 was strongly contaminated by bacteria. A semi-purified granule preparation was made by successive clarification of this material. First digestion of DNAs obtained from three samples using endonuclease Eco RI showed that DNAs from 1996 and 2001 standards were suitable for analysis by restriction enzymes whereas DNA extracted from the lyophilised powder was unsuitable.

In the following comparison, a *Carpocapsa pomonella* virus purified preparation dated 1971 was substituted for the oldest NPP virus. Different DNA obtained by the same rapid extraction method could not be cut by HindIII applying different buffers. This failure was attributed to the presence of an inhibitor present in the three DNAs.
The three viral DNAs (1971 virus, 2001 standard and 1996 standard) digested by 4 restriction enzymes (Eco RI, BamHI, SalI and EcoR V) were compared after separation on a gel.

Results:
Digested DNAs corresponding to NPP virus genomes named 1996 standard and 2001 standard did not show any difference in restriction profiles for visible fragments size range above 1000 nucleotides. The absence of differences was observed with 4 enzymes which offered spread and clearly visible profiles.
The two standards, 1996 NPP and 2001 NPP, could not be differentiated from a reference *Carpocapsa pomonella* granulovirus conserved at Saint Christol since 1971.

Conclusion:
According to the analysis criterion the purified 2001 standard virus from NPP, had not changed in comparison to the NPP 1996 standard. For the enzymes used, the NPP *standard of Carpocapsa pomonella* virus had the same profile as the *Carpocapsa pomonella* purified granulovirus conserved at Saint Christol (INRA) since 1971.

Reference:
Jehle, J., 2005. Comparative Restriction Analysis CpGV (CARPOVIRUSINE, technical concentrate, batch 2) with CpGV (INRA Mexican isolate) (BWS 2006-86)

The objective of the study was the comparison of CpGV (CARPOVIRUSINE, technical concentrate, batch 1461/SMT) with CpGV (INRA Mexican isolate). CpGV (INRA Mexican isolate) is the virus stock conserved at INRA of which the industrial production of CpGV (CARPOVIRUSINE, technical concentrate, batch 1461/SMT) is done. For the identification of both CpGV, DNA Restriction Endonuclease Analysis (REN) was used, with the endonucleases SalI, BamHI, EcoRI and EcoRV. The DNA enzyme profile of CpGV (CARPOVIRUSINE, technical concentrate, batch 1461/SMT) was compared with the DNA enzyme profile of CpGV (INRA isolate). In addition, both profiles were

compared with the profile known from the literature for CpGV-M (Crook et al. 1985, Crook et al. 1997).
It was found that the restriction profiles of both items did neither differ from each other nor to published restriction profiles of CpGV-M. It can be concluded that the test item CpGV (CARPOVIRUSINE technical concentrate, batch 1461/SMT) is identical to CpGV-M.

Material and methods:
Test item
Product: technical CpGV
Batch number: 1461/SMT
Number of CpGV (OB/mL), results from biological titration: $1.5 \times 10^{13} \pm 27$ %

Reference item
Product: CpGV INRA

Prior to DNA isolation the Test Item (CARPOVIRUSINE, technical concentrate, batch 1461/SMT) was purified. For the Reference Item (CpGV INRA Mexican isolate) no purification was necessary. The purified CpGV OB pellet was resuspended in 1 mL sterile water and the CpGV OB concentration was enumerated in the Petroff-Hausser counting chamber. The calculated number of CpGV OB/L was 4.03×10^{14}. The amount of the CpGV OB concentration of the Reference Item (CpGV INRA Mexican isolate) was enumerated in the Petroff-Hausser counting chamber. The calculated number of CpGV OB/L was 2.37×10^{15}. For the DNA isolation 5×10^{10} CpGV OB of the Test Item (CARPOVIRUSINE, technical concentrate, batch 1461/SMT) and of the Reference Item (CpGV INRA Mexican isolate) were used and isolated.
The viral DNA of the Test Item (CARPOVIRUSINE, technical concentrate, batch 1461/SMT) and of the Reference Item (CpGV INRA Mexican isolate) was digested with the restriction enzymes BamHI, EcoRI, SalI and EcoRV. For the Test Item (CARPOVIRUSINE, technical concentrate, batch 1461/SMT) 5 µL of the isolated DNA (about 650 ng DNA) was incubated with 2 µL buffer, 2 µL enzyme and 11 µL water (bidest.) at 37 °C for 3 h. Using the Reference Item (CpGV INRA Mexican isolate) 20 µL of the isolated DNA (about 650 ng DNA) was incubated with 2 µL buffer, 2 µL enzyme at 37 °C for 3 h. One tenth of each sample was used to proof if the digestion was successful as well as to compare the DNA concentrations. Starting from this information 17 µL of each digestion of the Test Item (CARPOVIRUSINE, technical concentrate, batch 1461/SMT) and 14 µL of the each digestion of the Reference (CpGV INRA Mexican isolate) were used for gel electrophoresis. All digested DNA were electrophoresed in a 0.8 % agarose gel over night (30 V) using TAE as a buffer system.

An updated restriction map of CpGV-M was provided Crook et al. (1997). They constructed a detailed restriction map of the in-vivo cloned genotype CpGV-M1. This cloned genotype did not differ from CpGV-M but was considered of not having any submolar heterogeneity caused by differing genotypes. CpGV-M1 was completely sequenced by Luque et al. (2001). The genome of CpGV-M1 is 1235000 bp. It has to be stressed that that CpGV-M1 cannot be considered to be identical with CpGV-M since it represents only a clone of CpGV-M and not all genotypes which potentially contribute to CpGV-M. However, since its restriction pattern is in accordance with the restriction pattern of CpGV-M (Crook et al., 1985; 1997) this sequence can be used as the best reference for CpGV-M, which is available in the moment. From the plain sequence DNA sequence the endonuclease restriction cutting sites can be predicted and the size of restriction fragments can be calculated.
Because no reference for EcoRV fragments of CpGV-M is available from literature, its sizes were deduced from the genome sequence of CpGV-M1 using the Genbank Acc.NC_002816 and applying the program Genequest (DNAStar).

Results:
The results of the different digests were as follows:

<u>SalI digest:</u>

All SalI restriction fragments A to W could be identified at the expected position. No submolar bands could be observed indicating that both items contained a homogenous virus preparation.

BamHI digest:
All BamHI restriction fragments A to N could be identified at the expected position. No submolar bands could be observed indicating that both items contained a homogenous virus preparation.
EcoRV digest:
All EcoRV restriction fragments A to L could be identified at the expected position. Fragments M and N were too small to be identified. Submolar bands could be observed for the test item CpGV (CARPOVIRUSINE, technical concentrate, batch 1461/SMT) and were indicated by asterisks. Since the other digests did not show any signs of heterogeneity these submolar fragments resulted most likely from an incomplete digest of the viral DNA rather than heterogeneity of the virus itself.

EcoRI digest:
All EcoRI restriction fragments A to N could be identified at the expected position. No submolar bands could be observed indicating that both items contained a homogenous virus preparation.

The reference item CpGV (INRA Mexican isolate) and the test item CpGV (CARPOVIRUSINE, technical concentrate, batch 1461/SMT) showed the identical restriction patterns for all four enzymes SalI, EcoRI, and BamHI. In addition, these restriction patterns are identical to the published patterns of CpGV-M and CpGV-M1, respectively. The EcoRV digest resulted in corresponding restriction pattern as to be expected from the genomic sequence of CpGV-M1. This indicates clearly that the reference item CpGV (INRA Mexican isolate) and the test item CpGV (CARPOVIRUSINE, technical concentrate, batch 1461/SMT) correspond to CpGV-M.

Such analysis is conducted by Arysta/NPP manufacturing plant every 2 to 4 years. (Analysis already conducted: Croizier, 1996; Biache, 1998; Croizier, 2001 and Jehle, 2005)

Conclusion by RMS
The study is still considered acceptable.

New information for RAR
The additional isolate CpGV R5 (GV-0007) from Arysta LifeSience S.A.S. was included in Annex III of the Review Report. A method for identification of this isolate is described in Vol. 3 MA B.1

B.5.1.2 Methods for providing information on possible variability of seed stock/active micro-organism

Andermatt Biocontrol GmbH
See Vol. 4 Andermatt

Arysta LifeScience S.A.S
Information already presented in the DAR
The genetic stability of CpGV amplified in the plants is regularly checked.

Statement by applicant for renewal
The information submitted previously is still valid and considered sufficient to cover current requirements. The manufacturing process has not changed and quality control measures are still the same as during original approval of CpGV.

B.5.1.3 Methods to differentiate a mutant of the micro-organism from the parent wild strain

Not relevant since used CpGV isolates are neither a mutant nor a genetically modified microorganism.

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

Andermatt Biocontrol GmbH

See Vol. 4 Andermatt

Arysta LifeScience S.A.S

See Vol. 4 Arysta

B.5.1.5 Methods to determine the content of the micro-organism in the manufactured material used for the production of formulated products and methods to show that contaminating micro-organisms are controlled to an acceptable level

Andermatt Biocontrol GmbH

Information already presented in the DAR

The granules (occlusion bodies) of CpGV are counted under the light microscope. The virus titre in the end-use product is adjusted to the requested granules/L.

Granule counts: The virus titre in a purified GV suspension can be determined by direct counting under a light microscope. Since the size of the virus granules is at the limit of light microscopic resolution, it is necessary to use dark field or phase contrast observation and the most powerful magnification possible (40-fold lens magnification). The disadvantage of a very restricted depth of focus can be compensated to some extent by the use of a counting chamber with a depth of 0.02 mm (Huber, 1981).

The concentration of active *Cydia pomonella* Granulovirus (CpGV) is determined by means of a quantitative bioassay.

The quantitative bioassay is performed with each batch of the end product before release. The bioassay is performed according to Andermatt (2004).

Statement by applicant for renewal

The information submitted for original approval of CpGV is still valid and considered sufficient to cover current data requirements. No new data are submitted.

Conclusion by RMS

A validated method is missing to determine the content of CpGV in technical MPCA in terms of granules/L or a description is missing how the content in terms of granules/L is derived from the bioassay tests.
The biological activity of CpGV can be determined by quantitative bioassay. Validation data for determination of biological activity are described in the study Walter, D. (2008) for the product MADEX. (see Vol. 3 MP Madex B.5.1.5). This method is considered also applicable for technical CpGV with adaptions due to the different concentration.

Arysta LifeScience S.A.S

Information already presented in the DAR

Technical concentrate analysis method:
The granules can be seen by light microscopy and can be counted by dark field microscopy, using an appropriate counting chamber as a Petroff Hauser counting chamber.

An estimation of the number of viable GV per volume unit in the Technical Concentrate of CARPOVIRUSINE is made by biological titration, carried out by comparison with a standard reference.

Determination of the concentration of CpGV
Reference:
Bonhomme, A. (2005), Procedure of the biological titration of batches at NPP (BWS 2006-100)

Knowing that the total number of OB counted in a batch by microscopy is not correlated to the activity of the virus inside the batch, it was judged necessary to express the concentration of CpGV correlated to their activity. The concentration of CpGV is therefore determined in every produced industrial batch of technical concentrate of CARPOVIRUSINE, by biological titration, carried out by comparison with a purified reference standard prepared at NPP and whose activity was checked to be comparable to published data. The details of the procedure is given below:

The number of viable occlusion bodies in a batch is estimated relatively to the biological activity of a purified reference standard whose number of OB has been determined by dark field microscopy and whose activity (LC_{50}) is validated by comparison with literature data.

1. Count the purified virus standard reference using dark field microscopy and a Petroff Hauser counting chamber 10 µm depth.
2. Make dilution series for the standard and for the batches to be titrated.
3. Make the Bioassay: prepare the artificial diet for each of the 96 well plates with the appropriate solutions of virus and put the neonate larvae in individual holes. Incubate for 10 days at 25 °C. Assess the dead and live larvae under binocular.
4. Make dose-response lines using probit analysis after naturally occurring mortality correction using the method of Abbott's formula.
5. Calculate the LD_{50} (*lethal dilution*) for the standard reference and for each batch using the software Genstat.

6. Calculate the relative potency (RPs) for each batch:

$$RPs = \frac{LD50 \quad reference}{LD50 \quad batch}$$

7. Calculate the biological titration of each batch:

$$batch \quad titer \quad = \frac{count \quad of \quad virus \quad reference \quad (OBs \quad / \, ml \quad of \quad solution \quad)}{RPs}$$

8. Final validation: assess the LC_{50} (expressed as OB/mL of diet) of the standard compared to literature data with the following calculation :

$$LC50 = \frac{count\ of\ virus\ reference}{LD\ 50}$$

If the LC_{50} of the virus standard reference is within the published range [2400 – 3300] OB/mL of diet, the bioassay is considered as acceptable. If not, the bioassay has to be reconducted.

Statement by applicant for renewal
The information submitted for original approval of CpGV is still valid and considered sufficient to cover current data requirements. No new data are submitted.

Conclusion by RMS
A validated method is missing to determine the content of CpGV in technical MPCA in terms of granules/L or a description is missing how the content in terms of granules/L is derived from the bioassay tests.
The biological activity of CpGV can be determined by quantitative bioassay. Validation data for determination of biological activity are described in the studies Wahl-Ermel/Jehle/ Eberle (2011) and Wahl-Ermel/Jehle (2012) for the product Carpovirusine. (see Vol. 3 MP Carpovirusine B.5.1.1). This method is considered also applicable for technical CpGV with adaptions due to the different concentration.

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

The data point is not relevant for a baculovirus, including CpGV, as they are not producing metabolites.

B.5.1.7 Methods to control the absence and to quantify (with appropriate limits of determination) the possible presence of any human and mammalian pathogen

Andermatt Biocontrol GmbH

Information already presented in the DAR
The contamination by other micro-organisms of each batch of the formulated product MADEX is analysed by an independent and certified laboratory.

For further information see Vol. 4 Andermatt and Vol. 3MP Madex B.5.

Arysta LifeScience S.A.S

Information already presented in the DAR
Each batch of the technical concentrate of CARPOVIRUSINE and of the formulated product CARPOVIRUSINE is checked for *Bacillus cereus* (PCA medium / colony count by pour plate method), which corresponds almost entirely to total flora.

Representative set of batch samples of technical concentrate of CARPOVIRUSINE and of the formulated product CARPOVIRUSINE are periodically sent to a microbiology lab and checked for microbial contaminants.

For further information see Vol. 4 Arysta and Vol. 3MP Carpovirusine B.5.

Serbios srl

Information already presented in the DAR
The formulated products are regularly checked for microbial contaminants.

For further information see Vol. 4 Serbios and Vol. 3MP Virgo B.5.

Statement by all applicants for renewal

For renewal of CpGV under Regulation (EC) 1107/2009 microbial contaminant screenings according to SANCO/1216/2012 rev. 0. have been carried out in the representative formulations. ISO methods were applied which are considered validated as such.

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

No EU data requirement.

B.5.2 Methods to determine and quantify residues (viable or non-viable) of the active micro-organism

Cydia pomonella GV is included in Annex IV of Regulation (EC) No 396/2005. Consequently, no maximum residue levels are set in food and feed. Also no action levels and no residue definitions are proposed or exist for *Cydia pomonella* GV in soil, water and air. Consequently, analytical methods for the determination of residues are not considered necessary.

B.5.2.1 The active micro-organism(s) on and/or in crop, in foodstuffs and feeding stuffs, in animal and human body tissues and fluids, in soil, in water (including drinking water, ground water and surface water) and in air where relevant (i.e. viable residues)

No residue definition is applicable for *Cydia pomonella* GV. Therefore, no post-registration monitoring methods are required.

Table B.5.2-1: List of additional methods for microorganisms, which are not considered to be essential

Author(s) and year	Report No	Reason
Biache, G., 1998 MET2006-329		A detection method of *Cydia pomonella* GV in soil samples is reported. Due to the lack of validation data the study is considered to be not acceptable.
Matt-Schmid, A.; Jehle, J., 2005 MET2006-239	GAB06	The study reports on the validation of an analytical method for determination of *Cydia pomonella* GV in soil. Due to insufficient recovery and repeatability data the method is not acceptable according to SANCO/825/00 rev. 8.1.
Jehle, J., 2004 MET2005-558	CPGV/2004-01	The study reports on the validation of an analytical method for the determination of *Cydia pomonella* GV in surface water. The study is not acceptable according to SANCO/825/00 rev. 8.1 due to insufficient recovery and repeatability data.
Biache, G., 1998 MET2006-328	MG/SC N° 1098	The protocol reports on the determination of residues of carpovirusine in apples and pears. No validation data are reported.
Matt-Schmid, A., 2005 MET2006-240	GAB04	The study reports on the validation of an analytical method for the determination of *Cydia pomonella* GV in water. The study is not acceptable according to SANCO/825/00 rev. 8.1 due to insufficient recovery and repeatability data.
Jehle, J., Matt-Schmid, A., 2006 ASB2013-5634	ARY04	Validation of an analytical method for the determination of *Cydia pomonella* GV in surface water. The study is not acceptable according to SANCO/825/00 rev. 8.1 due to insufficient recovery and repeatability data.

B.5.2.2 Relevant metabolites (especially toxins) on and/or in crop, in foodstuffs and feeding stuffs, in animal and human body tissues and fluids, in soil, in water (including drinking water, ground water and surface water) and in air where relevant (i.e. non -viable residues)

As *Cydia pomonella* GV is not known to produce any human pathogens, no analytical methods are required.

B.5.3 References relied on

Data point	Author(s)	Year	Title Owner, Report No. Source (where different from owner) GLP or GEP status Published or not BVL registration number	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previously submitted Y/N* If Y => old data point
KMA 4.1	Harvey, J.P., Volkman, L.E.	1983	BIOCHEMICAL AND BIOLOGICAL VARIATION OF CYDIA POMONELLA (CODLING MOTH) GRANULOSIS VIRUS not available, not applicable Virology, 124, 21-34 GLP/GEP: no Published: yes BWS2003-103	no	no	not protected	-	Y KIIM 4.1
KMA 4.1	Croizier, G.	2001	CARPOCAPSA POMONELLA GRANULOSIS VIRUS ANALYSIS. NPP 1996 STANDARD AN NPP 2001 STANDARD Arysta LifeScience S.A.S., EP01630 Institut National de la Recherche Agronomique, France GLP/GEP: no Published: no BWS2006-83	no	no	not protected	ALS	Y KIIM 2.10
KMA 4.1	Jehle, J.	2006a	COMPARATIVE RESTRICTION ANALYSIS CPGV (CARPOVIRUSINE, TECHNICAL CONCENTRATE, BATCH 1461/SMT) WITH CPGV (INRA MEXICAN ISOLATE) Arysta LifeScience S.A.S., ARY02 not available GLP/GEP: no Published: no BWS2006-86	no	no	not protected	ALS	Y KIIM 1.4.3.1

Data point	**Author(s)**	**Year**	**Title** **Owner, Report No.** **Source (where different from owner)** **GLP or GEP status** **Published or not** **BVL registration number**	**Vertebrate study Y/N**	**Data protection claimed Y/N**	**Justification if data protection is claimed**	**Owner**	**Previously submitted Y/N*** **If Y => old data point**
KMA 4.1	Bonhomme, A.	2006	PROCEDURE OF THE BIOLOGICAL TITRATION OF BATCHES AT NPP Arysta LifeScience S.A.S., not stated Natural Plant Protection, Pau GLP/GEP: no Published: no BWS2006-100	no	no	not protected	ALS	Y KIIM 1.4.1