Renewal Assessment Report

Cydia pomonella GV

Virgo

Volume 3 – B.5 Analytical methods

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Rapporteur Member State: Germany

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Version history

When	What
16 October 2020	First version submitted to EFSA

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.

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B Summary, evaluation and assessment of the data and information

B.5 Analytical methods

B.5.1 Methods for the analysis of the preparation

B.5.1.1 Methods for the identification and the determination of the content of the micro-organism(s) in the preparation

Data highlighted in grey has been included in DAR for *Cydia pomonella* Granulovirus (CpGV) Mexican Isolate (December 2007) Volume 3, Annex B-5, Point B 5.2 for VIRGO

Reference:
Fifi (2005), Bioassay for determination of the active *Cydia pomonella* Granulovirus by diet incoproduction toxicity to *Cydia pomonell*a, MAG032/01, Serbios srl (CHE2006-542)

Quantitative bioassay:
Principle of the methods:
The procedure is applicable to samples of formulated CpGV or to the active substance. A toxic standard must be included in the test series as a means of assuring that the laboratory test conditions are adequate and have not changed significantly. The toxic standard is an internal reference of known biopotency. Neonate larvae of *Cydia pomonella* are grown on a semisynthetic insect diet with various test substance concentrations. Several concentrations of the test substance are examined. After six days at 26 °C and 60 % relative humidity, the dead larvae are counted. Dose-mortality responses (LC50-values) are determined by probit analysis.

Conclusion by RMS
The study is still considered acceptable. Validation data for quantitative bioassay are described in the study Fifi (2006) (see below B.5.1.5). The methods are similar.
A validated method is missing to determine the content of CpGV in VIRGO in terms of granules/L or a description is missing how the content in terms of granules/L is derived from the bioassay tests.

B.5.1.2 Methods to establish regular control of the preparation to show that it does not contain other organisms than the indicated ones and to establish uniform

Information already presented in the DAR for VIRGO

The amount of active viruses in a batch is tested in all production batches by a quantitative bioassay.
For each production batch of the technical material the quantity of microbial load is documented. The presence of the human and/or mammalian pathogens (*Salmonella* spp., *Staphylococcus aureus* and Coliforms) is tested in the technical product. Only pathogen-free material is used for formulation of the end-product.

B.5.1.3 Methods to identify any contaminating micro-organisms of the preparation

The methods for determination of contaminating micro-organism in the formulation are described according to following published methods:

Table B.5.1-1: Microbial contaminants methods in VIRGO

Microbial contaminations and pathogens	Guideline methods
Bacillus cereus	NF EN ISO 7932:2005
Coliforms	NF EN ISO 4832:2006
Staphylococcus aureus	NF EN ISO 6888-3:2003
Salmonella spp.	NF EN ISO 6579:2002 ISO 6579:2002/Amd 1:2007
Aerobic plate count	MFLP-44 April 1998

Bacillus cereus (ISO 7932:2005)
Samples are plated on MYP (Mannitol-egg yolk-polymyxin) agar. After incubation at 30°C for 18 - 24 h, the *Bacillus cereus* colonies are counted.
Additionally, characteristic colonies are biochemically identified after plating on TSA and are also tested for haemolysis with sheep blood agar.

Coliforms (ISO 4832:2006)
Samples are incubated with violet red bile agar. After incubation at 37 °C for 24 h, the dishes are observed for characteristic and non-characteristic colonies.

Staphylococcus aureus (ISO 6888-3:2003)
After enrichment the solution is plated in petri dishes filled with Baird Parker agar. After incubation at 37 °C for 24 -48 h, the plates are observed for presence or absence of *Staphylococcus aureus.*

Salmonella spp. (ISO 6579:2002/Amd 1:2007)
Samples are suspended in buffered peptone water for pre-enrichment and incubated at 37 °C for 18 h. Afterwards, parts of the solution is incubated on MSRV (Rappaport –Vassiliadis modiefied) at 41 °C for 24 + 24 h. After incubation, the plates are observe to detect characteristic colonies.

Aerobic plate count (MFLP-44 April 1998)
Samples are plated on nutrient agar. After incubation at 30 °C for 72 h, the number of colony forming units are counted.

B.5.1.4 Methods for the determination of relevant impurities or metabolites in the manufactured material

Bacillus cereus is regarded as a relevant impurity in the formulation. With Regulation (EU) No 880/2014 the content of *Bacillus cereus* in the formulated product was set to $< 1 \times 10^7$ CFU/g.

Analytical method for *Bacillus cereus*, see B.5.1.3.

B.5.1.5 Methods used to determine the storage stability and shelf life of the preparation

References:
Fifi (2005), Physical, chemical, technical properties and shelf life of Virgo Cydia pomonella granulosis virus (2x10Exp13 GV/L, SC) (BVL no 3545715)
Fifi (2006), Physical, chemical, technical properties and shelf life of Virgo Cydia pomonella granulosis virus (2x10Exp13 GV/L, SC) at room temperature for one year (BVL no 3545714)

The method was used for the storage stability tests for 4 weeks at 40 °C and for 12 months at room temperature. It is in principle the same method as described above in B.5.1.1

Method
The diet - incorporation insect consists of eight serial dilutions where doses are quantified in terms of granulovirus (GV) per mL of diet. Three replicates of each sample of VIRGO were determined. The bioassay was considered valid if the corrected mortality in the untreated group was ≤ 17 %

Findings
The results of the probit analysis including the upper and lower 95 % limits were provided for each series of tests of Virgo.

B.5.2 Methods to determine and quantify residues (viable or non-viable)

All aspects with regard to the analytical methods of the product preparation are discussed in the context of the active substance in Volume 3 MA B.5.

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
KMP 5.1	Fifi, A.	2005	BIOASSAY FOR DETERMINATION OF THE ACTIVE CYDIA POMONELLA GRANULOVIRUS BY DIET INCORPORATION TOXICITY TO CYDIA POMONELLA Sipcam S.p.A., MAG032/01 Biotecnologie BT Srl, Fraz. Pantalla, Italy GLP/GEP: no Published: no CHE2006-542	no	no	not protected	SIP	Y KIIIM 5.1.3
KMP 5.1	Fifi, A.P.	2005	PHYSICAL, CHEMICAL, TECHNICAL PROPERTIES AND SHELF LIFE OF VIRGO CYDIA POMONELLA GRANULOSIS VIRUS (2X10EXP13 GV/LT, SC) Sipcam S.p.A., BT014/05 Biotecnologie BT Srl, Fraz. Pantalla, Italy GLP/GEP: no Published: no 3545715	no	no	not protected	SIP	Y KIIIM 2.2
KMP 5.1	Fifi, A.	2006	PHYSICAL, CHEMICAL, TECHNICAL PROPERTIES AND SHELF LIFE OF VIRGO CYDIA POMONELLA GRANULOSIS VIRUS (2X10EXP13 GV/L, SC) AT ROOM TEMPERATURE FOR ONE YEAR Sipcam S.p.A., BT015/05 Biotecnologie BT Srl, Fraz. Pantalla, Italy GLP: yes Published: no 3545714	no	no	not protected	SIP	Y KIIIM 2.2