Renewal
Assessment Report

Cydia pomonella GV

Carpovirusine

Volume 3 – B.5 Analytical methods

Rev. 0 – 16 October 2020

Rapporteur Member State: Germany

Co-Rapporteur Member State: The Netherlands

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

Information was already provided in the DAR

The CpGV content expressed as an estimated number of viable OB is checked by biological titration for each industrial batch of technical concentrate of CARPOVIRUSINE and also for the formulated product CARPOVIRUSINE.

The details of the biological titration procedure is given under point B.5.1.

For method details, see MA B.5

References:
Wahl-Ermel/Jehle/ Eberle (2011), Storage stability of Carpovirusine, ARY06 (BVL no 3545752)
Wahl-Ermel/Jehle (2012), Storage stability of Carpovirusine, ARY07 (BVL no 3545751)

Quantitative bioassay:
Principle of the method
The determination of the lethal dilution (LD_{50}) of Carpovirusine was done in bioassays. Neonate larvae of *Cydia pomonella* are grown on a semisynthetic insect diet with various test substance concentrations. Several concentrations of the test substance and reference substance are examined. In order to exclude first-day mortality of larvae from bioassay evaluation, the number of introduced larvae to the bioassay is reduced by the number of dead larvae. Thus only the surviving of day 1 are counted as introduced test animals. Mortality is scored again at day 9, the end of the bioassay. Dose-mortality responses (LD_{50}-values) are determined by probit analysis.

Findings
The respective equations of probit analysis and fiducial limits were provided for each series of tests for Carpovirusine and the reference CpGV.

Conclusion by RMS
The method is acceptable for the determination of the biological activity of CpGV in Carpovirusine.
A validated method is missing to determine the content of CpGV in Carpovirusine 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 was already provided 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 the 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.

Methods used for contaminating micro-organism are described in B.5.1.3

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 as stated by the applicant:

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

	Methods
Total flora (aerobes mesophilic)	XP V 08-034
Yeasts	NF V 08-059
Moulds	NF V 08-059
Bacillus cereus	NF EN ISO 7932
Salmonella	AES 10/04-05/04 (EN ISO 16140)
Staphylococcus coagulase positive (Staphylococcus aureus)/g at 37 °C	NF EN ISO 6888-2
Thermotolerant coli forms	NF V 08-060
Total coliforms at 30°C	NF V 08-050
Escherichia coli b glucoronidase positive	NF ISO 16649-2

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:
Wahl-Ermel/Jehle/ Eberle (2011), Storage stability of Carpovirusine, ARY06 (BVL no 3545752)
Wahl-Ermel/Jehle (2012), Storage stability of Carpovirusine, ARY07 (BVL no 3545751)

Bioassay for determination of *Cydia pomonella* was used for storage stability tests for 12 months at 4º C and 24 months at -18 ºC. For evaluation of the method, see B.5.1.1.

Reference:
Besse (2014), Storage microbiological stability of two batches of Carpovirusine at 25°C during 1 month, at 4°C during 8 months and at -18°C during 12 months (BVL no 3545790)

Determination of aerobic mesophilic flora

Aerobic mesophilic flora is determined at each sampling time for the two different Carpovirusine batches according to the French official method AFNOR XP V 08-034 method (surface seeding and colony count at 30°C by spiral method).

Determination of *Bacillus cereus*
The standard method NF EN ISO 7932 was used (horizontal method for presumptive count of *Bacillus cereus* at 30°C).

Conclusion by RMS
The methods were used for storage stability tests for 12 months at -18 °C, 4 °C or 25 °C. They are acceptable for the determination of aerobic mesophilic flora and *Bacillus cereus*.

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	Wahl-Ermel, B., Jehle, J., Eberle, K.	2011	STORAGE STABILITY OF CARPOVIRUSINE Arysta LifeScience S.A.S., ARY06 Dienstleistungszentrum Ländlicher Raum, Neustadt an der Weinstraße GLP: yes Published: no 3545752	no	yes	New data for new formulation, not previously submitted nor evaluated	ALS	N
KMP 5.1	Wahl-Ermel, B., Jehle, J.	2012	STORAGE STABILITY OF CARPOVIRUSINE Arysta LifeScience S.A.S., ARY07 Dienstleistungszentrum Ländlicher Raum, Neustadt an der Weinstraße GLP: yes Published: no 3545751	no	yes	New data for new formulation, not previously submitted nor evaluated	ALS	N
KMP 5.1	Besse, S.	2014	STORAGE MICROBIOLOGICAL STABILITY OF TWO BATCHES OF CARPOVIRUSINE AT 25°C DURING 1 MONTH, AT 4°C DURING 8 MONTHS AND AT -18°C DURING 12 MONTHS Arysta LifeScience S.A.S., 14/03 Natural Plant Protection, Pau GLP/GEP: no Published: no 3545790	no	yes	New data for new formulation, not previously submitted nor evaluated	ALS	N