Renewal Assessment Report

Cydia pomonella GV

Madex

Volume 3 – B.5 Analytical methods

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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 (KMP 5.1)

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

This study was already evaluated in the DAR:

Reference:
Andermatt, M. (2004), MADEX: Analysis of the Active ingredient (CHE 2006-685)
Anonymous (year of publication not stated): MADEX – Biotest zum Nachweis der Wirksamkeit eines MADEX Batches (CHE 2006-687)

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

Quantitative bioassay:
Principle of the methods:
Since 1988, Andermatt Biocontrol AG has kept a standard batch with a virus content of 3 x 10^{13} granules/L and since then, tested all production batches (formulated product) in comparison with this standard batch. Viruses are mixed up with a synthetic medium, so that following concentration grades can be tested: 2.5 x 10^2, 5 x 10^2, 2 x 10^3, 4 x 10^3 and 1 x 10^4 viruses per g medium. 50 g semisynthetic medium are weighed up in small plastic bags. The demanded amount of virus suspension is added from the dilution. Neonate larvae (L1) of *Cydia pomonella* are grown on the semisynthetic insect medium with the test substance concentrations. After 2-3 weeks at 25 °C the larvae are in the fifth, respectively in a last instar. The number of the survived larvae per concentration grade is counted. Through probit analysis the dose-mortality curve can be calculated. The new batch has passed the bioassay if its LD_{50} is not significantly higher than the one of the standard.

The difference between the above described method (Andermatt 2004, CHE 2006-685) and the method described in Anonymous (CHE 2006-687) is the concentration of the standard batch. In Anonymous (CHE 2006-687) the standard concentration is 1.5 x 10^{13} granules/L instead of the now used 3 x 10^{13} granules/L.

Conclusion by RMS
The study is still considered acceptable. Validation data for quantitative bioassay are described in the study Walter, D. (2008) (see below B.5.1.5). The methods are similar.
A validated method is missing to determine the content of CpGV in MADEX in terms of granules/L or a description is missing how the content 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:
The total germ count of each batch of MADEX is analysed by an independent and certified laboratory. The total germ count has to be less than 1 x 10^7 cfu/mL. Additionally the absence of *E. coli*, *S. aureus* and *Salmonella* spp. is verified.

The methods used for the determinations of pathogenic micro-organisms are typical microbiological

techniques with pathogen specified agars used in microbiological working laboratories. The methods used are described below 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:

Table B.5.1-1: Analytical methods for microbial contaminants in MADEX

Microbial contaminations and pathogens	Guideline methods
Aerobic mesophile count	ISO 4833
Bacillus cereus	ISO 7932
Staphylococcus aureus	ISO 6888-2
Escherichia coli	ISO 16649-2
Salmonella spp.	ISO 6579

aerobic, mesophile contaminants (ISO 4833)*:*
Known amounts of test substance have been mixed with liquid nutrient agar (plate count agar). After aerobic incubation for 3 days at 30 °C the colony forming units have been counted.

Bacillus cereus (ISO 7932:2004)
Samples are plated on MYP (Mannitol-egg yolk-polymyxin) agar. *B. cereus* colonies are counted after incubation at 30 °C.

Staphylococcus aureus (ISO 6888-2)*:*
Known amounts of the test substance have been plated on a selective solid nutrient agar (Rabbit plasma fibrinogen agar). After the aerobic incubation for 18-24 h at 37 °C the typical coloured colonies have been counted.

Escherichia coli (ISO 16649)*:*
Known amounts of test substance have been mixed on petri dishes with a liquid selective nutrient agar (Tryptone bile glucoronic agar). After the incubation for 18-24 h at 44 °C the typical blue coloured colonies have been counted.

Salmonella spp (ISO 6579):
Known amounts of the test substance have been incubated in a non selective nutrient agar for 16-20 h at 37 °C. After that a second enrichment step with two selective liquid agars (Brilliant green agar, Triple sugar iron agar) have been followed for 18-24 h at 42 °C. After the incubation the typical coloured colonies have been 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

Reference
Walter, D. (2008), Final report: Madex - 3.5 year storage stability at 5°C (BVL no 3545737)
The method was used for the 3.5 year storage stability test at 5 °C. It is in principle the same method as described above in B.5.1.1

Principle of method
The biological activity of CpGV is determined in a bioassay test. To calculate the dose-response dependent mortality, concentrations of virus were diluted in semi synthetic insect medium, for establishing the rate producing 50 % morality of neonate *Cydia pomonella* larvae (LD_{50}). The reference batch of ~~the virus~~ MADEX was run in parallel. Seven points from 0 µL to 500 µL were determined each. The dose-mortality curve was calculated through probit analysis.

Two units per treatment each containing 25 freshly hatched larvae were used. The bioassay units were stored in a climatic chamber at 25 °C ± 2 °C until the first L5 larvae appeared. The temperature of the climatic chamber and condition of the larvae were checked daily (except weekends).

Findings

Plots and the respective equations of probit analysis were provided for each series of tests for test and reference batch of MADEX.

Precision:
For determination of precision five replicates of the reference batch of MADEX were analysed.
One outlier had to be eliminated.

Mean of LD_{50} (4 replicates): 77.5 µL
Relative Standard deviation: 36.5%

For the 4 replicates lower 95% fiducial limits of LD_{50} ranged from 19 µL to 75 µL and the upper 95 % fiducial limits ranged from 66 µL to 150 µL.

Conclusion by RMS
The method is considered acceptably validated for determination of the biological activity of MADEX..

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

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	Andermatt, M.	2004	MADEX: ANALYSIS OF THE ACTIVE INGREDIENT Andermatt Biocontrol GmbH / Probis GmbH, not applicable Andermatt Biocontrol GmbH, Germany GLP/GEP: no Published: no CHE2006-685	no	no	not protected	PKA	Y KIIIM 5.1.3
KMP 5.1	Anonymous	1900	THE BIOASSAY US USED FOR THE DETECTION OF EFFICACY OF A MADEX BATCH (GERMAN ORIGINAL) Andermatt Biocontrol GmbH / Probis GmbH, not applicable not applicable GLP/GEP: no Published: no CHE2006-687	no	no	not protected	PKA	Y KIIIM 5.1.3
KMP 5.1	Walter, D.	2008	Final report: Madex - 3.5 year storage stability at 5°C Andermatt Biocontrol AG, CH, 20041161/01-PCTY eurofins-GAB GmbH, Niefern-Öschelbronn, Germany GLP: yes Published: no 3545737	no	yes	New data for existing formulation, not previously submitted nor evaluated	ABA	N