Renewal
Assessment Report

Cydia pomonella GV

Volume 3 – B.3 Data on application

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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 of the data and information

B.3 Data on application

B.3.1 Function

Viral entomopathogen, functioning as a microbiological insecticide.

B.3.2 Field of use envisaged

Orcharding and home-gardening

B.3.3 Crops or products protected or treated

According to the intended uses listed in documents D-1 of the individual formulated products, *Cydia pomonella* granulovirus is used in pome fruit (apple, pear, quince, nashi, *Mespilus*), stone fruit (peach, apricot, nectarine, almond, plum) and walnut against the codling moth (*Cydia pomonella*) and the oriental Fruit moth (*Grapholita molesta*).
Cydia pomonella granulovirus containing products are widely authorised in various European countries. For details please refer to documents D-2 of the individual company

B.3.4 Method of production and quality control

CONFIDENTIAL Information, please refer to documents J - Part A of the individual company.

B.3.5 Information on the occurrence or possible occurrence of the development of resistance of the target organism(s)

After intensive revision of the information presented by the applicant in Document MMA section 3 part 3.5, it is concluded that no further information on the development of resistance is necessary. The applicant provided sufficient and extensive information on the subject. Therefore, evaluation of the current status of resistance development in *Cydia pomonella* against different strains of CpGV is in full agreement with the applicant. However, it has to be mentioned here, that resistance is different according to the CpGV strain under evaluation. The former authorisation of the active substance was restricted to the Mexican isolate, however, the present renewal assessment report claims authorisation of *Cydia pomonell*a granulovirus without defining any particular strain. Four reference products have been submitted for evaluation, three of them consist of CpGV-M, the Mexican isolate, one, Madex-Twin, consist of the strain CpGV-V22. The latter possesses the same genome type as CpGV-M and is supposed to be used for control of oriental fruit moth (*Grapholita molesta*) according to the reference product dossier of MADEX TWIN (Andermatt Biocontrol AG). Up to date, no resistant populations of *Grapholita molesta* against CpGV-V22 has been reported.
For detailed information on resistance development against CpGV, please read the following text of the applicant:

Details of the mode of action of *Cydia pomonella* granulovirus are given in Point B.2.2.2. *Cydia pomonella* granulovirus (CpGV) acts highly specific against larvae of apple codling moth, *Cydia pomonella*, as a biological stomach insecticide. After infection of the midgut epithelium, other tissues are invaded, e.g. fat body, epidermis, tracheal matrix and Malphigian tubules. Rapid virus multiplication within the host cells finally results in cell destruction and at the end leads to lysis of the whole organism. The micro-organism is not translocated to other parts of the plant.

In the last years, single cases of reduced efficacy of CpGV-M formulations for the control of *Cydia pomonella* were reported. First observations on reduced susceptibility of *Cydia pomonella* populations to CpGV-M were made in Southern Germany in 2002 (Fritsch et al., 2006). While about half a dozen of orchards were affected by resistant *C. pomonella* by the end of 2005, this number doubled in 2006 and further cases were reported from Italy, Switzerland, and France (Fritsch et al., 2006; Sauphanor et al., 2006).

Resistant and susceptible populations were collected from different sites in Germany and reared in the laboratory. Resistance was maintained through several generations without any apparent change. In laboratory experiments, LD_{50}-values differed by a factor of 1000 between resistant and susceptible laboratory and field strains. Variations within the resistant populations are higher than in the susceptible populations which is apparent from the slope of the regression curve (Fritsch et al., 2006). This indicates a larger genetic variation in the resistant field populations when compared to the laboratory strain. So far, resistance is apparently independent from a history of preceding treatments with CpGV-M.

Sauphanor et al. (2006) isolated CpGV-M resistant *C. pomonella* populations from the south-east of France. These populations were found in plantations treated for longer periods with CpGV-M, but not with chemical insecticides. Susceptibility between laboratory strains and resistant populations differed by a factor of 13000 in the laboratory biotests. Resistance against CpGV-M is independent from resistance against chemical insecticides (Schmitt et al. 2008). There is also no cross-resistance to *Bacillus thuringiensis* Cry1AB insecticidal protein, on which many bioinsecticides are based (Eberle et al., 2008).

The genetic bases for resistance were analysed using traditional genetic methods. Initial crossing experiments to obtain information on the inheritance of resistance were performed by Eberle and Jehle (2006). Field resistance was stable even under laboratory conditions without selective pressure. The resistant strain showed 100 times lower susceptibility in 7-day bioassays. Offspring from mass crossing experiments between susceptible and resistant populations was analysed in bioassays. The median lethal concentration for the F1 progeny was intermediate between those of its parental strains. Reciprocal crosses suggested in the first instance that resistance is autosomally inherited. Results from backcrosses between F1 and the susceptible strain further indicated that inheritance of resistance is due to a non-additive, polygenic trait. Asser-Kaiser et al. (2007) further analysed the inheritance of resistance, because bioassays results of the progeny of single-pair crosses between resistant (CpR) and susceptible (CpS) *C. pomonella* individuals were heterogeneous, indicating that CpR populations still contained some susceptible individuals. Therefore, resistant populations were inbred and further selected using CpGV-M. The resulting genetically homogenous *C. pomonella* strain was termed CpRR1 and used together with the susceptible strain CpS for the analysis of inheritance of resistance. These experiments revealed that the resistance is linked to the sex chromosome Z (ZZ in males, ZW in females). Therefore, females with only one allel conferring resistance already have the full phenotypic resistance. This led to a rapid spread of resistance in the populations were resistance occurred. Quantitative estimation of resistance revealed that the gene-dose affects resistance to different CpGV levels. The heterozygous males are dominant at low virus pressure, which further enhances a rapid initial selection. At high virus concentrations, heterozygous males are recessive, leading to a selection of homozygous resistant males. Selection with CpGV-M would thus promote the resistance gene in the heterozygous state at low virus pressure. If virus rates are increased as a response of the farmer to reduced efficacy of the CpGV-M treatment, the resistance gene in the homozygous state is selected for, leading to an enforcement of resistance in the *C. pomonella* population. The genetic homogeneity of CpGV products in Europe has further promoted the development of resistance, because all products finally selected the same resistant genotypes (Asser-Kaiser et al., 2007). By revaluating the data of the original crosses CpR x CpS and applying the Z-linked, monomodel (Asser et al., 2007) it was confirmed that inheritance of resistance in CpR could also been explained by a Z-chromosomal, monogenic trait (Asser et al., 2010)

Fritsch et al. (2008) analysed the development of resistance in laboratory populations. A resistant field population (1000-fold less susceptible than the susceptible laboratory population) was collected from an orchard and reared in the laboratory in the presence or absence of CpGV-M. The resistance was

stable in absence of the virus for 32 generations (2.5 years). From this point on, resistance continuously decreased, and at generation 42, insects were only 10-fold less susceptible to the virus when compared to the susceptible population. In the presence of the virus, resistance could be increased when compared to the initial orchard population, indicating that further use of CpGV-M will increase problems with resistance in *C. pomonella* populations.
It has to be pointed out that resistance to CpGV-M is still restricted to relatively few orchards. However, strategies are necessary to effectively control these populations and to prevent the spread of the resistance. Different new CpGV isolates are available that are able to break the resistance against CpGV-M (Jehle et al. 2006). CpGV-V15, CpGV-V03, CpGV-V01 and CpGV-R5 are isolates that are able to infest *C. pomonella* larvae that are resistant to CpGV-M. These isolates are thus a well adapted tool to manage resistance against CpGV-M.
A literature search according to EFSA (2011)[5] was conducted covering the last 10 years. The literature research was conducted on the Scopus database (Anonymous, 2016). The search combined search terms "baculovirus", "granulovirus", virus name and names of the plant protection products based on CpGV with the term "resistance". In addition, some terms (Net present value, Predictive value and related terms) were excluded to limit background noise generated by the search term "NPV", abbreviation of "nucleopolyhedrovirus". 17 references were selected as potentially relevant for the subject of resistance to baculovirus and were subjected to full text analysis. Of those, 2 reports were identified as non-relevant, and 15 reports were identified as relevant and included below.

Conclusion by RMS:
There is development of resistance to CpGV in *C. pomonella* treated broadly with plant protection products based on this baculovirus. The mechanisms were extensively studied and vast information is available. It is most likely that the occurrence of resistance is a resultant of not a single factor. Nevertheless, it is also well studied how to counteract the resistance, by using various virus isolates.

B.3.5.1 Information on the occurrence or possible occurrence of the development of resistance of the target organism(s) against isolate CpGV-V14 and CpGV-V45 in equivalence to isolate CpGV-M

RMS considers that relevant new information has been submitted by the applicant in Documents M-MA regarding the resistance of *Cydia pomonella* against CpGV isolates V14 and V45. Both isolates are of particular interest, since they break the resistance already developed against other isolates, i.e. CpGV-M, in certain populations of the butterfly in Europe. The applicant cites new references regarding the mechanisms of resistance development and heritage pathways that should be integrated in the RAR (Section B 3.5 and reference lists).

B.3.6 Methods to prevent loss of virulence of seed stock of the micro-organism

CONFIDENTIAL Information, please refer to Doc J Part A of the individual company.

B.3.7 References relied on

Data point	Author(s)	Year	Title Owner, Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previously sub-mitted Y/N* If Y => old data point
B 3.5	Fritsch, E., Und-orf-Spahn, K., Kienzle, J., Zebitz, C., Huber, J.	2006	Apfelwickler-Granulovirus: Unterschiede in der Empfindlichkeit lokaler Apfelwickler-Populationen. Mitteilungen aus der Biologischen Bundes-anstalt, 400:310 GLP: no Published: yes	No	No			No
B 3.5	Sauphanor, B., Berling, M., Tou-bon, J.F., Reyes, M., Delatte, J.	2006	Carpocapse des pommes cas de résistance aux virus de la granulose dans le Sud-Est Phytoma, 590:24-27 GLP: no Published: yes	No	No			no
B 3.5	Schmitt, A., Huber, J., Sauphanor, B., Jehle, J.A.	2008	*Cydia pomonella* zeigt keine Kreuzresistenz zwischen Apfelwicklergranulovirus und Insektiziden. Mitteilungen aus dem Julius-Kühn-Institut, 417:427 GLP: no Published: yes	No	No			no

Data point	Author(s)	Year	Title Owner, Report No. Source (where different from owner) GLP or GEP status Published or not	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
B 3.5	Eberle, K.E., Asser-Kaiser, S., Sayed, S.M., Nguyen, H.T., Jehle, J.A.	2008	Overcoming the resistance of codling moth against conventional Cydia pomonella granulovirus (CpGV-M) by a new isolate CpGV-I12 Journal of Invertebrate Pathology, 98:293-298 GLP: no Published: yes	No	No			no
B 3.5	Eberle, K.E., Jehle, J.A.	2006	Field resistance of codling moth against *Cydia pomonella* granulovirus (CpGV) is autosomal and incompletely dominant inherited. Journal of Invertebrate Pathology, 93:201-206 GLP: no Published: yes	No	No			no
B 3.5	Asser-Kaiser, S., Fritsch, E., Undorf-Spahn, K., Kienzle, J., Eberle, K.E., Gund, N.A., Reineke, A., Zebitz, C.P.W., Heckel, D.G., Huber, J., Jehle, J.A.	2007	Rapid Emergence of Baculovirus Resistance in Codling Moth Due to Dominant, Sex-Linked Inheritance Science, 317: 1916-1918 GLP: no Published: yes	No	No			no

Data point	Author(s)	Year	Title Owner, Report No. Source (where different from owner) GLP or GEP status Published or not	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
B 3.5	Fritsch, E., Undorf-Spahn, K., Huber, J.	2008	Stabilität der Resistenz des Apfelwicklers gegenüber dem CpGV mit und ohne Selektionsdruck. Mitteilungen aus dem Julius-Kühn-Institut, 417:427-428 GLP: no Published: yes	No	No			no
B 3.5	Jehle, J.A., Blissard, G.W., Bonning, B.C., Cory, J.S., Herniou, E.A., Rohrmann, G.F., Theilmann, D.A., Thiem, S.M., Vlak, J.M.	2006	On the classification and nomenclature of baculoviruses: A proposal for revision. Archives of Virology, 151: 1257-1266 GLP: no Published: yes	No	No			no
B 3.5	Anonymous	2016	Literature review report on biological properties GLP: no Published: no	No	No			no