Appendix to:
EFSA (European Food Safety Authority), 2022. Conclusion on the peer review of the pesticide risk assessment of the active substance Cydia pomonella granulovirus (CpGV). EFSA Journal 2022;20(10):7630, 25 pp. doi:10.2903/j.efsa.2022.7630
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Appendix A - List of end points for the active substance and the representative formulation

Identity, Biological properties, Details of uses, Further information, and Proposed Classification and Labelling

| Active microorganism: | Cydia pomonella granulovirus (CpGV) |
|-----------------------|--------------------------------------|
| Function (e.g. control of fungi): | Insecticide |
| Rapporteur Member State: | Germany |
| Co-rapporteur Member State: | The Netherlands |

Identity of the Microbial or Viral Agent used in plant protection / Active Substance ) (Regulation (EU) N° 283/2013, Annex Part B, point 1 )

| Name of the organism: | Cydia pomonella granulovirus |
|-----------------------|-----------------------------|
| Taxonomy: | Family: Baculoviridae  
Genus: Betabaculovirus |
| Species, subspecies, strain: | Cydia pomonella granulovirus  
Several isolates |
| Identification / detection: | The identity of the virus produce can be bioanalytically checked against the other isolates by  
- SDS-polyacrylamide-gel electrophoresis of the virus proteins  
- Restriction endonuclease analysis of viral DNA |
| Culture collection:          | Andermatt Biocontrol GmbH |
|-----------------------------|--------------------------|
|                             | All isolates are deposited in the German Collection of Microorganisms and Cell Cultures (DSMZ), Inhoffenstraße 7B, D-38124 Braunschweig, Germany. |
| Mexican isolate:            | Virus accession number: GV-0001 |
| CpGV-V01:                   | Virus accession number: GV-0003 |
| CpGV-V03:                   | Virus accession number: GV-0006 |
| CpGV-V15:                   | Virus accession number: GV-0013 |
| CpGV-V22:                   | Virus accession number: GV-0014 |
| CpGV-V14:                   | Virus accession number: GV-0015 |
| CpGV-V45:                   | Virus accession number: GV-0017 |
| Arysta LifeScience S.A.S.   | All isolates are deposited in the German Collection of Microorganisms and Cell Cultures (DSMZ), Inhoffenstraße 7B, D-38124 Braunschweig, Germany. |
| Mexican isolate:            | Virus accession number: GV-0002 |
| CpGV-R5:                    | Virus accession number: GV-0007 |

| Minimum and maximum concentration of the MPCA used for manufacturing of the formulated product (CFU; g/kg): | Andermatt Biocontrol GmbH |
|--------------------------------------------------|--------------------------|
| Nominal CpGV-M concentration: 6 x 10^{13} OB/L |                         |
| Nominal CpGV-V22 concentration: 6 x 10^{13} OB/L |                         |
| Nominal CpGV-V14 concentration: 6 x 10^{13} OB/L |                         |
| Nominal CpGV-V45 concentration: 6 x 10^{13} OB/L |                         |
| Arysta LifeScience S.A.S.                           |                         |
| Nominal CpGV concentration: 3.2 x 10^{13} OB/L |                         |
| Serbios s.r.l                                             |                         |
| No own isolate is produced.                           |                         |

| Identity and content of relevant impurities, additives, contaminating organisms in the technical grade of MPCA: | Bacillus cereus: < 1 x 10^7 CFU/g in the formulated products |
|-----------------------------------------------------------------------------------------------------------|------------------------------------------------------------|
| The analysis of contaminating microorganisms in commercially produced batches of the representative formulations ‘Carpovirusine’, ‘Madex’ and ‘Madex Twin’ comply with the requirements of SANCO/12116/2012 rev.0 (European Commission, 2012). |
| Data gap: Analysis of at least four additional batches of the representative formulation ‘Virgo’ for the determination of microbial contaminants according to SANCO/12116/2012 were not provided by the applicant Serbios s.r.l. . |

| Is the MPCA genetically modified; if so provide type of modification | Not applicable |

**Biological properties of the microorganism** * (Regulation (EU) N° 283/2013, Annex Part B, point 2)

* **Data gap**: A transparent evaluation of the additional studies added to the dossier following the updated literature search for the biological properties of CpGV.

| Origin and natural occurrence, | CpGV-M has been isolated from codling moth larvae on apple and pear |
trees found in Mexico (near Valle de Allende, Chihuahua). CpGV is ubiquitous in the environment. Its geographic distribution corresponds with the distribution of its hosts.

| Background level: | Not known |
|-------------------|-----------|
| Target organism(s): | Larvae of the codling moth *Cydia pomonella* (all CpGV isolates, i.e. CpGV-M, CpGV-V01, CpGV-V03, CpGV-V15, CpGV-V22, CpGV-V45, and CpGV-R5) larvae of the oriental fruit moth *Grapholita molesta* (CpGV-R5, CpGV-V22 and CpGV-V45). |
| Mode of action: | The mode of action of CpGV is a bi-phasic infection process of the larval stages of *C. pomonella* and *G. molesta*. After oral ingestion of viral occlusion bodies, the virus replicates in the midgut cells (primary infection) and then infection is spread via non-occluded viruses to other body tissues (secondary infection) leading to the insect’s death. The body of the insect liquefies, and the virus is released into the environment where it can infect other codling moth and oriental fruit moth larvae. First-instar larvae are more sensitive to infection, and the tolerance increases with age until reaching its maximum at the fourth stage. Some of the larvae with late infection continue to grow but, after having reached the fifth stage, do not manage to form pupae. |
| Host specificity: | CpGV acts specifically against larvae of the codling moth *Cydia pomonella* and some isolates can infest the oriental fruit moth *Grapholita molesta* and the plum fruit moth *Grapholita funebrana*. In addition, cross transmission experiments have revealed alternative hosts of the tortricid family of the Lepidoptera for CpGV. Not pathogenic to humans / mammals / plants. |
| Life cycle: | The natural route of infection is the peroral ingestion of viral occlusion bodies by larvae. In the alkaline environment of the midgut, the occlusion bodies dissolve rapidly and occlusion-derived virions (ODV’s) are released. The ODV’s pass through the peritrophic membrane of the midgut. After attachment to the microvilli of the midgut epithelium, the nucleocapsids enter the cell lumen. The nucleocapsids are transported to the nucleus, the viral DNA is released, and DNA expression and replication is initiated. Initial replication produces non-occluded virus particles. By exocytosis the newly formed virions get to the hemolymph and from there into various tissues of the organism. After cell lysis a large number of occluded CpGV is set free which are then capable of infesting new hosts. |
| Infectivity, dispersal and colonisation ability: | The persistence of CpGV on leaves/fruit is mainly limited by sunlight. The calculated half-lives of CpGV due to UV-irradiation range from 15 to 52 sunlight hours. However, while about 99% of CpGV is rather quickly inactivated, a small portion of CpGV persists much longer. |
| In soil CpGV will persist for longer periods than on leaves/fruit. In soil persistence of CpGV is mainly regulated by the soil pH: the lower the pH, the more rapidly the virus is inactivated. |
| Little is known regarding the persistence of CpGV in natural aquatic environments. It is supposed that the pH and the salt concentration of the water influence its stability. |
| CpGV can be stored for two years at 5-8°C without losing any activity, however, it loses its efficacy if stored at temperatures above 54°C for more than 14 days, indicating that CpGV will likely also persist in the environment at lower temperatures but not at higher temperatures. |
Humidity does generally not show a direct influence on viral stability. However, there may be an indirect influence by affecting chemical action on the virus and by increasing the inactivation rate by sunlight.

No information has been provided in regard to temperature range at which CpGV is capable of proliferating.

Dispersal of CpGV includes via small animals and birds (their faeces are able to contain infective viruses), predators, windblown dry soil and rain splash at canopy edges. Knowledge of the importance of such mechanisms is limited.

| Relationships to known plant, animal or human pathogens: | CpGV as well as all other baculoviruses are not related to any known plant, animal (other than arthropods) or human pathogen. |
|---|---|
| Genetic stability: | As judged from restriction endonuclease analyses comparing the same CpGV isolate propagated in different institutions over several years CpGV is genetically stable. Furthermore, the isolates used in different formulations for several years did not change genetically compared to the originally described Mexican isolate CpGV-M. |
| | In very rare cases, CpGV may acquire genes and transposable elements from its host. Horizontal gene transfer has been occurring frequently within baculoviruses and indicates a role for baculoviruses as vectors of horizontal DNA transfer between insects. However, these mutants are considered to be out-competed by the wildtype CpGV and do not establish in a mixture. Host DNA inserted into the viral genome is evidently not maintained in any viral population after several successive infection cycles. |
| | Thus, although it cannot be excluded that a single virus may contain host DNA sequences, the recorded stability of the CpGV genome provides evidence that these mutants are extremely seldom and do not establish during the production process. |
| Information on the production of relevant metabolites (especially toxins): | Viruses have no metabolism of their own and are therefore not able to produce secondary metabolites. |
| Resistance/ sensitivity to antibiotics / anti-microbial agents used in human or veterinary medicine: | Not applicable as viruses are not metabolically active and thus, do not produce antimicrobial substances. Furthermore, viruses are not sensitive to antibiotics or other antimicrobial drugs and, accordingly, cannot become resistant to these substances or spread resistance. |
Summary of uses supported by available data (Regulation (EU) N° 283/2013, Annex Part B, point 3; OECD IIM Point 3)*

| Crop and/or situation (a) | Member State or Country | Product name | F or I (b) | Pests or Group of pests controlled (c) | Preparation | Application | Application rate per treatment | PHI (days) (m) | Remarks |
|--------------------------|-------------------------|--------------|------------|--------------------------------------|-------------|------------|-------------------------------|---------------|---------|
|                          |                         |              |            |                                      | Type (d-f)  |            |                               |               |         |
|                          |                         |              |            |                                      | Conc. MPCA (i) | method kind (f-h) | range of growth stages & season (j) | number min-max (k) | Interval between application (min) | OB /hL min-max (l) | Water L/ha min-max | OB /ha min-max (l) |         |
|                          |                         |              |            |                                      |             |            |                               |               | OB /ha min-max |         |         |         |         |
|                          |                         |              |            |                                      |             |            |                               |               | OB /ha min-max |         |         |         |         |

*Please provide specific data for each column in the table.*
## Peer review of the pesticide risk assessment of the active substance Cydia pomonella granulovirus (CpGV)

### Pome fruit
- **EU:** CARP
- **OVIR USINE**
- **F:** Codling moth (Cydia pomonella) Oriental fruit moth (Grapholita molesta)
- **SC:** 1 × 10³ OB CpGV-M/L product
- **Foliar spray (tract or drawn):** BBCH 71-89
- **10 days:** 1 × 10¹² CpGV-M/hL
- **1000:** 1 × 10¹⁰ CpGV-M/ha
- **I:** The application rate of 1 L/ha corresponds to 0.1 L/hL in 1000 L water/ha or 0.7 L/ha LWA (leaf wall area)

### Stone fruit
- **EU:** CARP
- **OVIR USINE**
- **F:** Codling moth (Cydia pomonella) Oriental fruit moth (Grapholita molesta)
- **SC:** 1 × 10³ OB CpGV-M/L product
- **Foliar spray (Knapsack sprayer):** BBCH 71-89
- **10 days:** 1 × 10¹² CpGV-M/hL
- **1000:** 1 × 10¹⁰ CpGV-M/ha
- **I:** Max. tree height: 2 m; The application rate of 1 L/ha corresponds to 0.1 L/hL in 1000 L water/ha or 0.7 L/ha LWA (leaf wall area)

### Walnut
- **EU:** MADE
- **X**
- **F:** Codling moth (Cydia pomonella)
- **SC:** 3 × 10³ OB CpGV-M/L product
- **Foliar spray (tract or drawn):** Before first larvae hatch from eggs*(i)* (BBCH 71-89)
- **10 days:** 0.25 × 10¹² × 0.75 × 10¹² CpGV-M/hL
- **400-1200:** 0.3 × 10³ CpGV-M/ha
- **I:** The application rate of 0.1 L/ha corresponds to 0.0875 L/ha LWA (leaf wall area)

### Pome fruit
- **EU:** MADE
- **X**
- **F:** Codling moth (Cydia pomonella)
- **SC:** 3 × 10³ OB CpGV-M/L product
- **Foliar spray (Knapsack sprayer):** Before first larvae hatch from eggs*(i)* (BBCH 71-89)
- **10 days:** 0.25 × 10¹² × 0.75 × 10¹² CpGV-M/hL
- **400-1200:** 0.3 × 10³ CpGV-M/ha
- **I:** Max. tree height: 2 m; The application rate of 0.1 L/ha corresponds to 0.0875 L/ha LWA (leaf wall area)

### Non-professional use
- **EU:** CARP
- **OVIR USINE**
- **F:** Codling moth (Cydia pomonella) Oriental fruit moth (Grapholita molesta)
- **SC:** 1 × 10³ OB CpGV-M/L product
- **Foliar spray (tract or drawn):** BBCH 71-89
- **10 days:** 1 × 10¹² CpGV-M/hL
- **1000:** 1 × 10¹⁰ CpGV-M/ha
- **I:** Max. tree height: 2 m; The application rate of 1 L/ha corresponds to 0.1 L/hL in 1000 L water/ha or 0.7 L/ha LWA (leaf wall area)
### Peer review of the pesticide risk assessment of the active substance *Cydia pomonella* granulovirus (CpGV)

| Stone fruit (apricot, peach, nectarine, almond, plum) | **EU** | **MADE X TWIN** | **F** | Oriental fruit moth (*Grapholita molesta*) | **SC** | Foliar spray (tract or draw) | Before first larvae hatch from eggs (BBCH 71-89) | **12** | 6-8*2 | 0.375 × 10^{12} CpGV-V22/hL | 800 | 0.3 × 10^{13} CpGV-V22/ha | - | Non-professional use |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Stone fruit (apricot, peach, nectarine, almond, plum) | **EU** | **MADE X TWIN** | **F** n | Oriental fruit moth (*Grapholita molesta*) | **SC** | Foliar spray (Knap sack spray) | Before first larvae hatch from eggs (BBCH 71-89) | **12** | 6-8*2 | 0.375 × 10^{12} CpGV-V22/hL | 800 | 0.3 × 10^{13} CpGV-V22/ha | - | Non-professional use |
| Pome fruit (apple, pear, quince, nashi pears) | **EU** | **VIRGO** | **F** | Codling moth (*Cydia pomonella*) | **SC** | Foliar spray (tract or draw) | BBCH 71-87 | 6 | 7 | 0.1 × 10^{13} – 0.88 × 10^{13} CpGV-M/hL | 1500-1700* | 1.5 × 10^{13} CpGV-M/ha | 3 | Minimum dose rate: 0.5 L/ha; The application rate of 0.75 L/ha corresponds to 0.656 L/ha LWA (leaf wall area). |

# GAPs/representative use information was not available for the CpGV isolates: CpGV-V01 (Virus accession number: GV-0003), CpGV-V03 (Virus accession number: GV-0006), CpGV-V15 (Virus accession number: GV-0013), CpGV-V14 (Virus accession number: GV-0015), CpGV-V45 (Virus accession number: GV-0017) and CpGV-R5 (Virus accession number: GV-0007)

*1 First treatment 85 degree days after the first warm evening with flight activity. Zero point of development of the codling moth is 10°C.

*2 sunny days, counting 2 partially sunny days as 1 day

*3 The lower water volume should be used for lower trees, whereas the highest water amount is recommended for trees with a higher leaf area. In case of very expanded leaf area which requires more than 1500 L water/ha, a higher water volume can be applied, but the maximum rate of 15 × 10^{15} GV/ha must be respected.

(a) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (e.g. fumigation of a structure)

(b) Outdoor or field use (F), glasshouse application (G) or indoor application (I)

(c) e.g. biting and sucking insects, soil born insects, foliar fungi, weeds

(d) e.g. wettable powder (WP), emulsifiable concentrate (EC), granule (GR)

(e) CropLife International Technical Monograph no 2, 6th Edition. Revised May 2008, Catalogue of pesticide formulation types and international coding system.

(f) All abbreviations used must be explained

(g) Method. e.g. high-volume spraying, low volume spraying, spreading, dusting, drench

(h) Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plant - type of equipment used must be indicated

(i) CFU = colony forming units and g/kg or g/L

(j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application

(k) Indicate the minimum and maximum number of applications possible under practical conditions of use

(l) PHI - minimum pre-harvest interval

(m) Remarks may include: Extent of use/economic importance/restrictions
Classification and proposed labelling (Symbol, Indication of danger, Risk phrases, Safety phrases)

| With regard to physical/chemical data: | Not relevant |
|--------------------------------------|--------------|
| With regard to toxicological data:   | Not applicable to viruses. Commercial products containing the technical material by Andermatt/Serbios should be labelled as follows: ‘Contains *Cydia pomonella* granulovirus. Microorganisms may have the potential to provoke sensitising reactions’. The technical concentrate SMT-M and the formulation ‘Carpovirusine’ (both from Arysta) may trigger the criteria for classification under CLP Regulation (EC) No 1272/2008: **Skin Sensitising category 1** (based on exceedance of the specific concentrations limits (SCL) and on positive test with the formulation) |
| With regard to fate and behaviour:    | Not relevant |
| With regard to ecotoxicological data: | Not relevant |

**Methods of analysis** (Regulation (EU) № 283/2013, Annex Part B, point 4 and Regulation (EU) № 284/2013, Annex Part B, point 5)

**Analytical methods for the microorganism** (MA 4.1 & MP 5.1)

| Manufactured microorganism (principle of method): | Isolate identification by Restriction Fragment Analysis (RFLP) or single nucleotide polymorphism (SNP) Determination of the active ingredient by a standard bioassay with the target pest. |
|----------------------------------------------------|---------------------------------------------------------------------------------------------------|
| Impurities and contaminating microorganisms in manufactured material (principle of method): | Standard microbiological methods (EN ISO) for microbial contaminant screening including *Bacillus cereus* according to SANCO/12116/2012-rev.0 |
| Microbial Pest Control Product (principle of method): | See above, methods for the microorganism **Data gap**: A validated method to determine the content of CpGV isolate in the formulation ‘Virgo’ in terms of OB/L and a description on how the content in terms of OB/L is derived from the bioassay tests have not been provided by the applicant Serbios s.r.l. |

**Analytical methods for residues (viable and non-viable) in exposed compartments and organisms** (MA 4.2 & MP 5.2)

| Of the active microorganism (principle of method): | Methods are not required. |
|---------------------------------------------------|--------------------------|
| Of relevant metabolites (principle of method):     | Methods are not required. |
Impact on Human and Animal Health (Regulation (EU) N° 283/2013, Annex Part B, point 5 and Regulation (EU) N° 284/2013, Annex Part B, point 7)

| Medical data: (including medical surveillance on manufacturing plant personnel) (MA 5.1.1) | No evidence of adverse effects in humans, *Cydia pomonella* granulovirus is not related to human or animal pathogens. |
| Sensitisation: (MA 5.2.1 & MP 7.2.3) | No experimental data for the virus itself.  
- Representative formulation ‘Carpovirusine’: positive (M&K test)  
- Representative formulation ‘Madex’ and ‘Madex Twin’: unacceptable test  
- Representative formulation ‘Virgo’: negative (M&K test)  
According to Regulation (EU) N° 283/2013, all microorganisms should be considered potential sensitizers. |
| Acute oral infectivity, toxicity and pathogenicity: (MA 5.2.2.1 & MP 7.1.1) | No evidence of adverse effects, 
LD₅₀ > 1.015 x 10⁸ granules/animal (rat) |
| Acute intratracheal/inhalation infectivity, toxicity and pathogenicity: (MA 5.2.2.2 & MP 7.1.2) | No evidence of adverse effects, 
LC₅₀ > 2 x 10¹³ granules/L (rat, nose-only exposure, with representative formulation ‘Virgo’)  
According to Regulation (EU) N° 283/2013, all microorganisms should be considered potential sensitizers. |
| Acute intravenous/intraperitoneal infectivity: (MA 5.2.2.3) | No evidence of adverse effects, 
LD₅₀ > 1.015 x 10⁷ granules/animal (rat, intraperitoneal) |
| Genotoxicity: (MA 5.2.3) | Negative in studies of limited scientific value,  
Unlikely to be genotoxic based on general knowledge on baculoviruses. |
| Cell culture study: (MA 5.2.4) | CpGV penetrated into human W138 cells but did not replicate there, no transcription of viral genes was observed. |
| Information on short-term toxicity and pathogenicity: (MA 5.2.5) | No reliable data, not necessary. |
| Dermal toxicity: (MP 7.1.3) | No data, not necessary. |
| Specific toxicity, pathogenicity and infectivity: (MA 5.3) | No reliable data, not necessary. |
| Genotoxicity – *in vivo* studies in germ cells: (MA 5.5) | No data, not necessary. |

Reference values

| AOEL: | Not applicable to Baculoviruses |
| ADI: | Not applicable to Baculoviruses |
| ARfD: | Not applicable to Baculoviruses |

Exposure (operator, workers, bystander, consumer): (MA 6.1 & MP 7.3, 8.0)  
No health risk expected for operator, worker, bystander or residents for the intended uses. PPE is recommended for the operator with respect to the sensitising potential of micro-organisms (including viruses).

Residues (Regulation (EU) N° 283/2013, Annex Part B, point 6 and Regulation (EU) N° 284/2013, Annex Part B, point 8)  
Viable residues: Not relevant because of no concern for dietary exposure.
Non-viable residues: Non-viable residues (toxic metabolites or degradation products) do not occur because viruses do not produce metabolites, as they self-replicate within host organisms. QPS ‘qualified presumption of safety’ without safety qualifications/restrictions has been recommended to the family Baculoviridae which includes SeMNPV since 2009 until to date (EFSA BIOHAZ Panel, 2009<sup>1</sup> and 2022<sup>2</sup>).

Fate and Behaviour in the Environment (Regulation (EU) N° 283/2013, Annex Part B, point 7 and Regulation (EU) N° 284/2013, Annex Part B, point 9)

| Persistence and multiplication (competitiveness) in soil, water and air: | *Cydia pomonella* granulovirus (CpGV) belongs to the group of baculoviruses. Baculoviruses are ubiquitous in the environment, their prevalence depending on the frequency of occurrence of their arthropod hosts. Granuloviruses have to be considered as persistent in soil, as they are protected from UV-light in deeper soil layers. Multiplication can restart again if the permissive host appears. Granuloviruses precipitate quickly in an aquatic system at similar rates as soil particles. Transport into the sediment phase is likely. Activity in sediment remaining for a length of time similarly as in soil cannot be excluded. The virus is inactivated by sun light. A half-life of 52 hours was determined. Occlusion bodies of granuloviruses can be considered as suspended solid particles that are non-volatile. Therefore, a distribution of CpGV via air can be excluded except for any aerosols formed at the time of spraying. The following PEC<sub>soil</sub> values were calculated for the respective formulations: 13.96 mg ‘Carpovirusine’/kg soil (corresponding to 1.33 × 10<sup>8</sup> GV/kg soil); 1.548 mg ‘Madex’/kg soil (corresponding to 4.00 × 10<sup>7</sup> GV/kg soil); 1.858 mg ‘Madex Twin’/kg soil (corresponding to 4.80 × 10<sup>7</sup> GV/kg soil); 6.60 mg ‘Virgo’/kg soil (corresponding to 1.20 × 10<sup>8</sup> GV/kg soil). The following PEC<sub>SW</sub> values were calculated for the respective representative formulations: PEC<sub>SW</sub> of 302.3 µg ‘Carpovirusine’/L (corresponding to 2.89 × 10<sup>6</sup> GV/L); 33.5 µg ‘Madex’/L (corresponding to 8.66 × 10<sup>5</sup> GV/L); 40.2 µg ‘Madex Twin’/L (corresponding to 1.04 × 10<sup>6</sup> GV/L); 152 µg ‘Virgo’/L (corresponding to 2.76 × 10<sup>6</sup> GV/L). |
| Mobility: | Granuloviruses are able to leach through a column of soil. Results of a field lysimeter experiment conducted 1987 Germany indicate an acceptable low risk of reaching deeper soil layers and therefore the groundwater. The good retention of baculoviruses by soil is probably attributed to the particular protein envelope of the virus particles consisting |

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<sup>1</sup> EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2009. Scientific Opinion on the maintenance of the list of QPS biological agents intentionally added to food and feed (2009 update). EFSA Journal 2009;7(12)1431, 92 pp. https://doi.org/10.2903/j.efsa.2009.1431

<sup>2</sup> EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2022. Update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 16: suitability of taxonomic units notified to EFSA until March 2022. EFSA Journal 2022;20(7):7408 38 pp. DOI: https://doi.org/10.2903/j.efsa.2022.7408
of granulin. Dispersal of the virus particles by insects cannot be excluded. Also see entry above for infectivity, dispersal and colonisation ability regarding possible mobility via other organisms.

Effects on non-target organisms (Regulation (EU) N° 283/2013, Annex Part B, point 8 and Regulation (EU) N° 284/2013, Annex Part B, point 10)

### Effects on birds (MA 8.1 & MP 10.1)

| Application rate (kg MPCA/ha) | Test substance | Crop | Category (e.g. insectivorous bird) and species | Time-scale | Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects) |
|------------------------------|----------------|------|-----------------------------------------------|------------|-------------------------------------------------------------------------------------|
| max. total rate per crop and season: $1 \times 10^{14}$ GV/ha (overall worst-case) | CARPOVI RUSEINE | Pome fruit | Test species: Northern bobwhite *Colinus virginianus* | 30 days | NOEL and LD50 > 10,000 mg/kg bw/day, corresponding to $10^{11}$ granules/kg bw (5 days dosing period; resultant total dosage was 50,000 mg/kg over 5 days); No signs of toxicity, infectivity and pathogenicity. |

### Effects on mammals

| Application rate (kg MPCA/ha) | Test substance | Crop | Category (e.g. herbivorous mammal) and species | Time-scale | Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects) |
|------------------------------|----------------|------|-----------------------------------------------|------------|-------------------------------------------------------------------------------------|
| max. total rate per crop and season: $1 \times 10^{14}$ GV/ha (overall worst-case) | CARPOVI RUSEINE | Pome fruit | Test species: Rat (Sprague-Dawley) | 14 days (Post exposure observation period) | LD50 > 5,000 mg/kg bw, corresponding to $> 4.9 \times 10^{10}$GV/kg bw; No signs of toxicity, infectivity or pathogenicity. |

### Effects on other terrestrial vertebrates

| Application rate (kg MPCA/ha) | Test substance | Crop | Group (e.g. amphibian) and species | Time-scale | Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects) |
|------------------------------|----------------|------|---------------------------------|------------|-------------------------------------------------------------------------------------|
| No information provided. |

Risk assessment for birds and mammals for exposure via drinking water (worst-case among intended uses)

| Generic focal species | Body weight [kg] | Total water ingestion rate [L/day] | Maximum spray suspension | PEC [GV/L] | Daily dose [GV/kg bw] | Toxicity LD50 [GV/kg] | MOS |
|-----------------------|------------------|----------------------------------|-------------------------|------------|------------------------|------------------------|-----|
effects on aquatic organisms (MA 8.2 & 10.2)

| Group | Test substance | Time-scale | Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects) |
|-------|----------------|------------|----------------------------------------------------------------------------------|
| **Fish species:** | | | |
| *Danio rerio* | CARPOVIRUSINE | 96 hours | LC_{50} > 250 mg/L, corresponding to 2.08 \times 10^9 GV/L; No signs of toxicity/infection/pathogenicity |
| *Oncorhynchus mykiss* | MADEX * (= Granulosevirus CpGV SC) | 96 hours | LC_{50} > 100 mg/L, corresponding to 1.9 \times 10^6 GV/L; No signs of toxicity/infection/pathogenicity |
| *Oncorhynchus mykiss* | VIRGO | 96 hours | LC_{50} > 100 mg/L, corresponding to 1.61 \times 10^6 GV/L; No signs of toxicity/infection/pathogenicity |
| **Invertebrate species:** | | | |
| *Daphnia magna* | CARPOVIRUSINE | 48 hours | EC_{50} > 250 mg/L, corresponding to 2.08 \times 10^9 GV/L; No signs of toxicity/infection/pathogenicity |
| *Daphnia magna* | MADEX * (= Granulosevirus CpGV SC) | 48 hours | EC_{50} > 100 mg/L, corresponding to 1.9 \times 10^6 GV/L; No signs of toxicity/infection/pathogenicity |
| *Daphnia magna* | VIRGO | 48 hours | EC_{50} > 100 mg/L, corresponding to 1.61 \times 10^6 GV/L; No signs of toxicity/infection/pathogenicity |

* tested as ‘Granupom’. The two formulations ‘Granupom’ (2.2 x 10^{13} granules/L) and ‘Madex’/‘Madex Twin’ (3 x 10^{13} granules/L) contains nearly the same amount of granules/L. Therefore, their comparability is considered as sufficient.

Effects on algae: (species, growth, growth rate, capacity to recover) (MA 8.2.3 & MP 10.2)

| | |
|---|---|
| Unicellular green alga (*Pseudokirchneriella subcapitata*); Test substance: ‘Carpovirusine’; EC_{50} > 100 mg/L, corresponding to 8.3 \times 10^6 GV/L; no signs of toxicity, infectivity or pathogenicity; |
| Green alga (*Scenedesmus subspicatus = Desmodesmus subspicatus*); |
Test substance ‘Madex’ * (= Granulosevirus CpGV SC); EC50 > 100 mg/L, corresponding to $1.9 \times 10^9$ GV/L; no signs of toxicity, infectivity or pathogenicity

Unicellular green alga (Pseudokirchneriella subcapitata): Test substance: ‘Virgo’; EC50 > 100 mg/L, corresponding to $1.61 \times 10^9$ GV/L; no signs of toxicity, infectivity and pathogenicity

**Effects on aquatic plants**

| Species | Test substance | Time scale/type of endpoint | End point | Toxicity |
|---------|----------------|-----------------------------|-----------|----------|
| Gibbous duckweed (Lemna gibba) | ‘Madex’ * (= Granulosevirus CpGV SC) | acute | oral toxicity 48h (LD50) | $> 1.1 \times 10^6 \mu g$ CpGV/bee ($> 108.4 \mu g$ product/bee) |
| | | | contact toxicity 48h (LD50) | $> 1.1 \times 10^6 \mu g$ CpGV/bee ($> 100 \mu g$ product/bee) |
| Common duckweed (Lemna minor) | ‘Virgo’ | acute | oral toxicity 48h (LD50) | $> 1.63 \times 10^6 \mu g$ CpGV/bee ($> 100 \mu g$ product/bee) |
| | | | contact toxicity 72h (LD50) | $> 1.63 \times 10^6 \mu g$ CpGV/bee ($> 100 \mu g$ product/bee) |
| | ‘Madex’ | acute | oral toxicity 72 (LD50) | $> 3.5 \times 10^7 \mu g$ CpGV/bee |
| | ‘Madex Twin’ | acute | contact toxicity 48h (LD50) | $> 4.4 \times 10^7 \mu g$ CpGV/bee |

CpGV: *Cydia pomonella* granulovirus

* tested as ‘Granupom’. The two formulations ‘Granupom’ (2.2 x $10^{13}$ granules/L) and ‘Madex’/‘Madex Twin’ (3 x $10^{13}$ granules/L) contain nearly the same amount of granules/L. Therefore, their comparability is considered as sufficient.

**Effects on bees (Regulation (EU) N° 283/2013, Annex Part A, point 8.3.1 and Regulation (EU) N° 284/2013 Annex Part A, point 10.3.1)**

| Species | Test substance | Time scale/type of endpoint | End point | Toxicity |
|---------|----------------|-----------------------------|-----------|----------|
| Apis mellifera L. | CARPOVIRUSINE | acute | oral toxicity 48h (LD50) | $> 1.1 \times 10^6 \mu g$ CpGV/bee ($> 108.4 \mu g$ product/bee) |
| | | | contact toxicity 48h (LD50) | $> 1.1 \times 10^6 \mu g$ CpGV/bee ($> 100 \mu g$ product/bee) |
| | VIRGO | acute | oral toxicity 72h (LD50) | $> 1.63 \times 10^6 \mu g$ CpGV/bee ($> 100 \mu g$ product/bee) |
| | | | contact toxicity 72h (LD50) | $> 1.63 \times 10^6 \mu g$ CpGV/bee ($> 100 \mu g$ product/bee) |
| | ‘Madex’ | acute | oral toxicity 72 (LD50) | $> 3.5 \times 10^7 \mu g$ CpGV/bee |
| | ‘Madex Twin’ | acute | contact toxicity 48h (LD50) | $> 4.4 \times 10^7 \mu g$ CpGV/bee |

Potential for accumulative toxicity: no data

Infectiveness and pathogenicity: To investigate the infectiveness and pathogenicity several studies have been generated by a literature search and was evaluated. No toxic, infective or pathogenic effects were observed.
Further information*:
The reliable information from the literatures regarding the impact on honey bee colonies was tested in two field studies (Cantwell 1966; Knox 1970) and one laboratory study (Gröner 1978). The results do not indicate any harmful effects on colony development or bee mortality. No signs of an impact of brood development in bumble bee colonies could be detected in another study (Mommaerts et al., 2009). It was reported that the host range of granuloviruses appears to be narrow and mostly restricted to a single species. Furthermore, the high host specificity of CpGV to only a few species of Tortricidae family (Lepidoperta) was also reported (for more information refer to Volume 3 – B.2 Biological properties). Altogether, the risk for bee larvae or, in general, for the bee brood could be assumed as negligible. Information on data already evaluated are re-evaluated and reported.

* A new study on bumble bees with the product ‘Granupom’ (active substance: Cydia pomonella granulovirus, concentration $2.2 \times 10^{13}$ CFU/g) was submitted (B.9.3.1/1 (Mommaerts et al., 2009). However, as the study was considered only as supportive information no summary is listed here.

### Effects on terrestrial arthropods other than bees (MA 8.4 & MP 10.4)

| Species                  | Stage | Test Substance | Dose (kg MPCA/ha) | Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects) |
|--------------------------|-------|----------------|-------------------|------------------------------------------------------------------------------------------|
| **Laboratory Tests**     |       |                |                   |                                                                                          |
| *Hippodamia convergens* | adult | CARPOVIRUS SINE | 55 - 550 - 5500 ppm treatment group; administered by honey | 30 day-LC$_{50}$ > 5500 ppm; 30 day-NOEC ≥ 5500 ppm (corresponding to $5.5 \times 10^{10}$ GV/g food); no signs of toxicity/infectivity/pathogenicity |
| *Chrysoperla carnea*     | larva | CARPOVIRUS SINE | 55 - 550 - 5500 ppm treatment group; administered in a moth egg diet | 10 day-LC$_{50}$ > 5500 ppm; 10 day-NOEC ≥ 5500 ppm (corresponding to $5.5 \times 10^{10}$ GV/g food); no signs of toxicity/infectivity/pathogenicity |
| *Aphidius rhopalosiphi*  | adult | CARPOVIRUS SINE | 37 – 111 – 333 – 1000 – 3000 mL/ha; extended Laboratory Study using | 48 hour-LR$_{50}$ > 3000 mL/ha; 48 hour-ER$_{50}$ > 3000 mL/ha; no signs of toxicity/infectivity/pathogenicity |
### Peer review of the pesticide risk assessment of the active substance *Cydia pomonella* granulovirus (CpGV)

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| Insect Species | Neoteny Stage | Active Substance | Test Conditions | Toxicity/Infectivity/Pathogenicity |
|---------------|---------------|------------------|----------------|-----------------------------------|
| *Typhlodromus pyri* | proto-nymphs | CARPOVIRUSINE | treated barley seedlings/food | 7 day-LR$_{50}$ > 3000 mL/ha; 7 day-ER$_{50}$ > 3000 mL/ha; no signs of toxicity/infectivity/pathogenicity |
| *Aphidius rhopalosiphi* | proto-nymphs | MADEX * (= Granulosevirus CpGV SC) | 360 mL/ha (Limit test); exposure to treated glass plates | 48 hour-LR$_{50}$ > 360 mL/ha; 48 hour-ER$_{50}$ > 360 mL/ha; (corresponding to $7.92 \times 10^{12}$ GV/ha); no signs of toxicity/infectivity/pathogenicity; Given the specific mode of action, exposure scenario via treated glass plates (without treated food) not suitable to detect possible effects of CpGV on *Aphidius rhopalosiphi* |
| *Typhlodromus pyri* | proto-nymphs | MADEX * (= Granulosevirus CpGV SC) | 360 mL/ha (Limit test); exposure to treated glass plates | 7 day-LR$_{50}$ > 360 mL/ha; 7 day-ER$_{50}$ > 360 mL/ha; (corresponding to $7.92 \times 10^{12}$ GV/ha); no signs of toxicity/infectivity/pathogenicity; Given the specific mode of action, exposure scenario via treated glass plates (without treated food) not suitable to detect possible effects of CpGV on *Typhlodromus pyri* |
| *Poecilus cupreus* | proto-nymphs | MADEX * (= Granulosevirus CpGV SC) | 360 mL/ha (Limit test); exposure to treated quartz sand/food | 14 day-LR$_{50}$ > 450 mL/ha; 14 day-ER$_{50}$ > 450 mL/ha; (corresponding to $9.9 \times 10^{12}$ GV/ha); no signs of toxicity/infectivity/pathogenicity |
| *Aphidius rhopalosiphi* | proto-nymphs | VIRGO | 1725 mL/ha (Limit test); exposure to treated glass plates | 48 hour-LR$_{50}$ > 1725 mL/ha; 48 hour-ER$_{50}$ > 1725 mL/ha; (corresponding to $3.45 \times 10^{13}$ GV/ha); no signs of toxicity/infectivity/pathogenicity; Given the specific mode of action, exposure scenario via treated glass plates (without treated food) not suitable to detect possible effects of CpGV on *Aphidius rhopalosiphi* |
| *Typhlodromus pyri* | proto-nymphs | VIRGO | 1725 mL/ha (Limit test); exposure to treated glass plates | 7 day-LR$_{50}$ > 1725 mL/ha; 7 day-ER$_{50}$ > 1725 mL/ha; (corresponding to $3.45 \times 10^{13}$ GV/ha); no signs of toxicity/infectivity/pathogenicity; Given the specific mode of action, exposure scenario via treated glass plates (without treated food) not suitable to detect possible effects of CpGV on *Typhlodromus pyri* |
* tested as ‘Granupom’. The two formulations ‘Granupom’ (2.2 x 10^{13} granules/L) and ‘Madex’/’Madex Twin’ (3 x 10^{13} granules/L) contains nearly the same amount of granules/L. Therefore their comparability is considered as sufficient.

**Effects on other terrestrial invertebrates (MA 8.5 & MP 10.5)**

| Test substance ‘Carpovirusine’: | Test substance ‘Granupom’ (≈ CpGV SC): |
|---------------------------------|----------------------------------------|
| Acute study with *Eisenia fetida*; 14 day-LC₅₀ > 1000 mg/kg soil dw; no signs of toxicity, infectivity and pathogenicity; Reproduction study with *Eisenia fetida*: 56 day-NOEC ≥ 1000 mg/kg soil dw; no signs of toxicity, infectivity or pathogenicity. | Acute study with *Eisenia fetida*: 14 day-LC₅₀ > 1000 mg/kg soil dw, corresponding to 1.67 x 10^{10} GV/kg soil dw; no signs of toxicity, infectivity or pathogenicity |

| Test substance ‘Virgo’: | Test substance ‘Granupom’ (≈ CpGV SC): |
|--------------------------|----------------------------------------|
| Acute study with *Eisenia foetida*: 14 day-LC₅₀ > 1000 mg/kg soil dw, corresponding to 1.61 x 10^{10} GV/kg soil dw; no signs of toxicity, infectivity or pathogenicity. | Acute study with *Eisenia foetida*: 14 day-LC₅₀ > 1000 mg/kg soil dw, corresponding to 1.67 x 10^{10} GV/kg soil dw; no signs of toxicity, infectivity or pathogenicity |

**Further information:**

Literature search did not indicate any adverse effects on earthworms associated with the use of baculoviruses.

**Effects on soil microorganisms (MA 8.6 & MP 10.6)**

All formulations tested (‘Carpovirusine’, ‘Granupom’ (as Granulosevirus CpGV SC), ‘Virgo’ had no negative impact on soil microorganisms. In conclusion, CpGV represents no hazard to soil microorganisms.

**Additional studies (MA 8.7 & MP 10.7)**

No additional studies have been conducted.