Renewal Assessment Report

Cydia pomonella GV

Virgo

Volume 3 – B.9 Effects on non-target organisms

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Rapporteur Member State: Germany

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Version history

When	What
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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.9 Effects on non-target organisms

In the following, for ease of presentation, data and their evaluations from the original DAR and addenda to the DAR are highlighted grey.

No new data were submitted for the renewal of the approval for VIRGO (*Cydia pomonella* Granulovirus (CpGV-M)).

VIRGO is used as a foliar spray for the control of Codling moth (*Cydia pomonella*) in pome fruits and walnut. A summary of the critical Good Agricultural Practice of VIRGO is presented in Table B.9.1-1.

Table B.9.1-1: Summary of intended uses for VIRGO

Crop and/ or situation	F G or I	Pests or Group of pests controlled	Application			Application rate per treatment		
			Method / Kind	Timing / Growth stage of crop & season	Max. number / min. interval between applications	L product / ha a) max. rate per appl. b) max. total rate per crop/season	GV / ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha/ mch min / max
Pome fruits and Walnut	F	Codling moth (*Cydia pomonella*)	Foliar spray (tractor drawn)	BBCH 71 - 87	6/7 days	a) 1.5×10^{13} GV/ha b) 9×10^{13} GV/ha	a) 0.75 b) 4.5	1500 - 1700*

*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 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^{12} GV/ha must be respected.

B.9.1 Effects on birds

The following information was already submitted in the DAR (2008) Volume 3, Annex B-9, Point 9.2.1: In general, it is referred to the information submitted for the active substance. The substances of the preparation VIRGO formulated as SC are inert and no hazards to birds are expected. Therefore, studies and information on the active substance are considered applicable and relevant with regard to the evaluation of the formulated product on birds. Furthermore, it has to be kept in mind that CpGV is highly specific to codling moth (*Cydia pomonella* (L.), Lepidoptera: Tortricidae) only.

B.9.1.1 Risk assessment for birds

In RMS' point of view, no quantitative risk assessment is deemed necessary for the following reasons:

- High selectivity: *Cydia pomonella* Granulovirus (CpGV) is highly specific and only has an effect on very few species of the Tortricidae family (Lepidoptera).
- There are no major deviations from the GAP uses previously assessed in the DAR (2008) with the exception of a slightly higher max. total rate per crop/season.
- As can be seen from the initial DAR (2008), risk quotients (Margin-of-Safety-values) clearly exceeded the default trigger values.
- Literature search submitted for the renewal of the approval for CpGV did not indicate any adverse effects on birds and mammals associated with the use of baculoviruses (see Anonymous, 2016, BVL no 3306490; data point KMA 8/01).

Nevertheless, a quantitative risk assessment for terrestrial vertebrates (birds and mammals) is provided below for illustrative purposes.

Effects on birds and mammals

No experimental data for VIRGO were submitted for the first approval of *Cydia pomonella* Granulovirus (CpGV) to address the pathogenicity and infectiveness to birds and mammals. In general, it is referred to the information submitted for the active substance (please refer to Doc M-MA, Section 8, Point MA 8.1 and Doc M-MA, Section 5, Point MA 5.2.2.1). The substances of the formulated product VIRGO are inert and no hazards to birds and mammals are expected (please refer to Doc J (SER), Part B, for Serbios srl). Furthermore, CpGV is highly specific to codling moth (*Cydia pomonella* (L.), Lepidoptera: Tortricidae) only. The family of baculoviruses, including CpGV, is regarded to be safe for humans and vertebrates (EFSA[1]). Additionally, the literature search provided covering the last 10 years revealed no new relevant information.

All available data for birds and mammals indicate that VIRGO is not toxic, not pathogenic or infective to birds or mammals. Nevertheless, a quantitative risk assessment based on the EU agreed endpoints confirming the safe use is provided.

The EU agreed endpoints are summarised in the following table.

Table B.9.1-1: Summary of the studies on effects on birds and mammals; toxicity and pathogenicity of *Cydia pomonella* Granulovirus (CpGV)

Test substance	Test species	Endpoint	Reference
CARPOVIRUSINE	Bobwhite quail	**NOEL = 10000 mg/kg bw** (equivalent to 1.0×10^{11} GV/kg bw)	EFSA Journal 2012;10(4):2655[2]
CARPOVIRUSINE	Rat, acute oral	LD_{50} > 5000 mg/kg bw ($LD_{50} > 4.9 \times 10^{10}$ GV/kg bw)	EFSA Journal 2012;10(4):2655[2]

The available endpoints for birds and mammals indicate no toxicity or pathogenicity of *Cydia pomonella* Granulovirus (CpGV). No effects on birds and mammals have been reported.

Exposure

Birds and mammals are typically exposed to dry spray deposits on their food items following the dilution and via drinking water following spraying of the formulated product. During spraying, much of the formulation constituents are likely to be lost by volatilisation. Therefore, where oral exposure is the main route of exposure, toxicity data for the active substance are used in preference to data from tests with the formulated material. Exposure via dermal and inhalation routes is considered unlikely, since at the time of application and for a short period thereafter, most wild birds and mammals will leave the immediate vicinity of spray operations in response to the human disturbance. Birds and mammals may be exposed directly and indirectly via the ingestion of sprayed plant parts and via infected arthropods, respectively.

The potential exposure of birds to CpGV was estimated following GAP directed applications of the product in the different uses at maximum application rates.

Risk Assessment - Birds and Mammals

For risk assessment for effects on birds and mammals the 'European Food Safety Authority Guidance Document on Risk Assessment for Birds and Mammals' (EFSA Guidance document 2009)[3] is available. However, this document in first line is compiled for the risk assessment of chemical substances. There-

[1] EFSA Journal 2015; 13(12):4331

[2] European Food Safety Authority; Conclusion on the peer review of the pesticide risk assessment of the active substance *Cydia pomonella* granulovirus. EFSA Journal 2012;10(4):2655

[3] European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12): 1438. [139 pp.].

fore, the risk assessment approach is not feasible for microbial substances as not only biological parameters of the birds and mammals go into calculations but also chemical properties, like K_{oc} values from the test item, 90th percentile residue values that come from a database for chemicals.
For the exposure via drinking water a risk assessment in accordance to SANCO 4145/2000[4] is presented, which is considered more appropriate and is considered to represent a worst-case.

Exposure via drinking water
Risk assessment to drinking water is performed in accordance with SANCO 4145/2000[4]. Species that frequent open water bodies are able to ingest spray deposits of active substances that reach water for example via spray drift from treated fields. The exposure density in this case is equal to PED_{sw}, calculated in Table B.9.2-5.

In some situations, some species may obtain all their daily water demand directly from puddles of spray liquid or reservoirs held in the axils of leaves. This situation can be considered as worst case. The exposure density can be calculated from the dilution used to prepare the product for spraying. Analysis has shown that initial densities in such sources are in the range 5 - 20% of the sprayed concentration, therefore a dilution factor of 5 is applied for the risk assessment.

Thus the PED_{puddle} is calculated as:
PED_{puddle} = maximum spray suspension density × 0.20

The daily water intake is calculated as follows:
Birds: Total water ingestion rate (L/day) = $0.059 \times W^{0.67}$
Mammals: Total water ingestion rate (L/day) = $0.099 \times W^{0.9}$
Where:
W = body weight in kg

Thus, the daily dose of active substance intake is calculated as

$$\text{Daily dose} = \frac{\text{PEDpuddle} \times \text{total water ingestion rate}}{W}$$

Where:
W = body weight in kg

The risk of *Cydia pomonella* Granulovirus (CpGV) to birds and mammals was assessed from margin of safety (MOS; corresponding to TER) values according to the following equation:

$$\text{MOS} = \frac{\text{LD}_{50}\ [\text{GV/kg bw}]}{\text{daily dose}\ [\text{GV/kg bw}]}$$

Based on the available data the MOS values of birds and mammals for CpGV were calculated as follows.

Table B.9.1-2: Risk assessment for birds and mammals for exposure via drinking water (puddles) following GAP directed application of VIRGO in orchards in accordance with SANCO 4145/2000[5]

Indicator species	Body weight [kg]	Total water ingestion rate [L/day]	maximum spray suspension concentration [GV/L]	PED_{puddle} [GV/L]	Daily dose [GV/kg bw]	Toxicity [a] LD_{50} [GV/kg bw]	MOS

[4] European Commision, Health & Consumer Protection Directory, Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC, SANCO/4145/2000 - final, 25 September 2002
[5] European Commision, Health & Consumer Protection Directory, Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC, SANCO/4145/2000 - final, 25 September 2002

Small insectivorous bird - tit, wren	0.010	0.002697	1.0×10^{10}	2.0×10^{9}	5.39×10^{8}	$> 1.0 \times 10^{11}$	> 185
Small herbivorous mammal - vole	0.025	0.003579			2.86×10^{8}	$> 4.9 \times 10^{10}$	> 171

a) The presented LD_{50} are "greater than" values. No lethal, sublethal or pathogenic effects have been observed at these highest rates tested.

Calculation of the exposure via water can be considered worst case. The density in the water is directly related to the spray application. In the drinking water risk assessment for birds and mammals the CpGV specific endpoints in GV/kg bw were used for the calculations. The resulting MOS values indicate that no adverse effects in birds and mammals are to be expected due to exposure to “contaminated” drinking water following GAP directed use of VIRGO.

Comments by the RMS (2020):

From the MOS-calculations presented above, a low risk for birds and mammals can be concluded, especially as no lethal, sublethal or pathogenic effects have been observed at the highest doses tested.

B.9.2 Effects on aquatic organisms

The following information, highlighted in grey, was already submitted in the DAR (2008) Volume 3, Annex B-9, Point 9.3.1, 9.3.2 and 9.3.3 and is now summarised in more detail.

B.9.2.1 Effects on fish

Plant protection product

Reference:	[redacted]2005): Acute toxicity testing of VIRGO in rainbow trout (*Oncorhynchus mykiss*) (Teleostei, Salmonidae); unpublished report no. 20051166/01-AAOm, BVL no 1300694
Guideline:	OECD Guideline 203 and Annex to commission Directive 92/69/F-EC, procedure C.1
GLP:	Yes
Material and methods:	
Test substance:	VIRGO; purity: 2×10^{13} GV/L
Test species:	*Oncorhynchus mykiss* (Walbaum); weight: not specified; length: from 4 to 6 cm
Number of test animals:	10 fish per concentration
Treatments:	0, 1, 10 and 100 mg/L
Duration:	96 hours
Test conditions:	Static system; Temperature: 15.0 - 16.6°C; Photoperiod: 12-hour photoperiod; Oxygen content: > 71% of the air saturation value; Hardness: 13°dH (231.4 mg/L as CoCO3); pH: 7.19 - 8.41; no food during the study
Deviations from guideline:	- One from ten measured fishes was larger than 3.5 cm. - Slight deviation of temperature was noted beyond the norms, with a minimum at 18.9°C. - Temperature variation was greater than ± 1°C in the control medium. These deviations were not considered to have affected the outcome or the objectives of the study.
Endpoint:	Survival, growth and behaviour

Observations:	Daily check for mortality, occurrence of sublethal effects (loss of equilibrium, erratic swimming loss of reflex, excitability, discolouration, or change in behaviour), dissolved oxygen, pH and temperature

Results:

Neither significant mortality nor sublethal effects were observed at any concentration up to the nominal concentration level of 100 mg/L over 96 h. No unusual observations of appearance or behaviour of fish were made.

A summary of endpoints is given in the table below.

Toxic effects / Infectivity / Pathogenicity of plant protection product to fish

Test species	*Oncorhynchus mykiss*
Toxicity of plant protection product	no signs of toxicity/infectivity/pathogenicity
	LC_{50} > 100 mg/L

Comments by the RMS (2019):

The study is acceptable.
The LC_{50} value at 96 h was estimated to be > 100 mg/L VIRGO. The NOEC is ≥ 100 mg/L.
As the test was performed according to the OECD guideline 203, there is no information concerning infectivity and pathogenicity. Due to the very narrow host specifity of CpGV, effects on fish populations are not expected. Additional information is not required.

B.9.2.2 Effects on freshwater invertebrates

Plant protection product

Reference:	Fifi, A. (2005a): Evaluation of VIRGO (CpGV 2x10^{13} GV/LT, SC) toxic effects on *Daphnia magna* using test of acute immobilisation; unpublished report no. BT007/05, BVL no 1300693
Guideline:	OECD Guideline 202 (1984), OECD Guideline: "Revised Proposal for Updating Guide-line 202", Daphnia sp., Acute Immobilisation Test, Revised Draft Document October 2000
GLP:	Yes
Material and methods:	
Test substance:	VIRGO; purity: 2 × 10^{13} GV/L
Test species:	*Daphnia magna*
Number of test animals:	20 per concentration
Treatments:	0, 1, 10 and 100 mg/L
Duration:	48 hours
Test conditions:	Static system; Temperature: 20.1 - 21.3°C; Photoperiod: darkness; Oxygen content: > 60% of the air saturation value; Hardness: 250 ± 25 mg CaCO3/L; pH: 6.5 - 8.5
Deviations from guideline:	None
Endpoint:	Immobility, mortality
Observations:	Daily check for mortality/immobilization, dissolved oxygen, pH and temperature

Results:

The results are reported in Table B.9.2-1.

Table B.9.2-1: Mortality of *Daphnia magna*

Concentration (mg/L)	Numbers of *Daphnia* exposed	Response at 24 h		Response at 48 h	
		Number immobile	%	Number immobile	%
Control	20	0	0	1	5
1.0	20	0	0	0	0
10	20	0	0	0	0
100	20	2	10	0	10

A summary of endpoints is given in the table below.

Toxic effects / Infectivity / Pathogenicity of plant protection product to freshwater invertebrates

Test species	*Daphnia magna*
Toxicity of plant protection product	no signs of toxicity/infectivity/pathogenicity
	EC_{50} > 100 mg/L

Comments by the RMS (2019):

The study is acceptable.
The 24 h and 48 h-EC_{50} value exceeded the concentration of 100 mg VIRGO/L. As the test was performed according to the OECD guideline 202, there is no information concerning infectivity and pathogenicity. Due to the very narrow host specifity of CpGV, effects on aquatic invertebrates are not expected. Additional information is not required.

B.9.2.3 Effects on algae growth

Plant protection product

Reference:	Fifi, A. (2005b): Determination Of Virgo (CpGV 2x10^{13} GV/lt, SC) toxic effects on the algal growth (*Pseudokirchneriella subcapitata*); unpublished report no. BT006/05, BVL no 1300692
Guideline:	OECD Guideline 201: Alga, Growth Inhibition Test and EEC Directive C.3, Alga inhibition test.
GLP:	Yes
Material and methods:	
Test substance:	VIRGO; purity: 2 × 10^{13} GV/L
Test species:	*Pseudokirchneriella subcapitata*
Number of test animals:	n.a. (initial cell density: 10^4 cells per mL)
Treatments:	0 and 100 mg/L (limit test)
Duration:	72 hours
Test conditions:	Static system; Temperature: 21 - 25°C; Photoperiod: Continuous illumination with a light intensity of 6000 to 10000 Lux with a maximum deviation of ± 15%; Test unit: Erlenmeyer flasks of 250 mL volume; pH: 7.96 - 8.02 at test start and 8.06 - 8.23 at test end
Deviations from guideline:	None.
Endpoint:	Biomass and growth rate

Observations:	Cell concentration for each flask at each measuring point (24, 48, 72 h), and method for measuring cell concentration, mean values of cell concentration, growth curves

Results:

No significant effects were detected at 100 mg/L. The EC_{50} values were estimated to be > 100 mg/L.

A summary of endpoints is given in the table below.

Toxic effects / Infectivity / Pathogenicity of plant protection product to algae

Test species	*Pseudokirchneriella subcapitata*
Toxicity of plant protection product	no signs of toxicity/infectivity/pathogenicity
	EC_{50} > 100 mg/L

Comments by the RMS (2019):

The study is acceptable.
On the basis of the observation made during this test, the EC_{50} was determined to be >100 mg/L and the NOEC was determined to be ≥ 100 mg/L.

B.9.2.4 Effects on plants other than algae

Plant protection product

Reference:	Fifi, A. (2005c): Toxic effects of VIRGO (CpGV 2x10^{13} GV/LT, SC) on the duckweed growth (*Lemna minor*); unpublished report no. BT012/05, BVL no 1300691
Guideline:	OECD Guidelines for the Testing of Chemicals, revised proposal for a new guideline 221, July 2002 “Lemna sp. growth inhibition test“:- Internal method MEC012 “Test di inibisione della crescita su Lemna minor”
GLP:	Yes
Material and methods:	
Test substance:	VIRGO; purity: 2 × 10^{13} GV/L
Test species:	*Lemna minor*
Number of test animals:	n.a. (initial frond number: 12)
Treatments:	0, 100 mg/L (limit test)
Duration:	7 days
Test conditions:	Semi-static system; Temperature: 24°C ± 2°C; Photoperiod: Continuous illumination with a light intensity of 6500 to 10000 Lux with a maximum deviation of ± 15%; Test units: Erlenmeyer flasks of 100 mL volume; pH 6.47 - 6.80 at test start and pH 6.99 - 7.18 at test end
Deviations from guideline:	None.
Endpoint:	Frond number, final fresh/dry weight, changes in development
Observations:	Frond numbers (normal and abnormal) and their appearance were determined at the beginning and end of the test. Counts of frond numbers were determined every two days. Changes in plant development (e.g. frond size, appearance, necrosis, chlorosis or gibbosity, colony break-up or loss of buoyancy, root length, morphology or breakdown). In addition effects of the test substance on final biomass are also assessed based on dry weight and fresh weight.

Results:

The toxic effects of VIRGO on *Lemna minor* are presented in Table B.9.2-2 (negative values mean growth promotion). The test results can be regarded to be valid, as doubling time of control frond numbers must be less than 2.5 days (60 h). A light increase of the final biomass after 7 days of exposure period was observed.

Table B.9.2-2: Toxic adverse of VIRGO on *Lemna minor* after application of 100 mg/L (based on limit test results)

Parameters	0 - 3 d	3 - 5 d	5 - 7 d	7 d
Growth rate (% inhibition)	-15.32	-26.44	-16.86	-18.60
Doubling times (d)	1.9	2.0	1.8	-
Biomass increase (% inhibition)	-	-	-	-19

A summary of endpoints is given in the table below.

Toxic effects / Infectivity / Pathogenicity of plant protection product to plants

Test species	*Lemna minor*
Toxicity of plant protection product	no signs of toxicity/infectivity/pathogenicity
	EC_{50} > 100 mg/L

Comments by the RMS (2019):

The study is acceptable.
On the basis of the observation made during this test, the EC_{50} was determined to be >100 mg/L and the NOEC was determined to be ≥ 100 mg/L.

B.9.2.5 Risk assessment for aquatic organisms

Table B.9.2-3: Summary of the studies on toxicity on aquatic organisms treated with toxin/metabolite from the active ingredient or the plant protection product VIRGO.

Species	Test duration	Dose range	Results/ Endpoint	Observations	Reference
Toxin/Metabolite	Not relevant as viruses do not produce secondary metabolites or toxins.				
Plant protection product					
Rainbow trout (*Oncorhynchus mykiss*)	96 hours	0, 1, 10 and 100 mg/L	LC50 > 100 mg/L, NOEC ≥ 100 mg/L	No signs of toxicity/pathogenicity	[redacted] (2005), BVL no 1300694
Water flea (*Daphnia magna*)	48 hours	0, 1, 10 and 100 mg/L	EC50 > 100 mg/L, NOEC ≥ 100 mg/L	No signs of toxicity/pathogenicity	Fifi, A. (2005a), BVL no 1300693
Green algae (*Pseudokirchneriella*	72 hours	0 and 100 mg/L (limit test)	EC50 > 100 mg/L,	No signs of toxicity/pathoge	Fifi, A. (2005b), BVL no

Species	Test duration	Dose range	Results/ Endpoint	Observations	Reference
subcapitata)			NOEC ≥ 100 mg/L	nicity	1300692
Duckweed (*Lemna minor*)	7 days	0 and 100 mg/L (limit test)	EC50 > 100 mg/L, NOEC ≥ 100 mg/L	No signs of toxicity/pathogenicity	Fifi, A. (2005c), BVL no 1300691

In RMS' point of view, no quantitative risk assessment is deemed necessary given the lack of toxicity, infectivity or pathogenicity from laboratory data in conjunction with the following available information:

- High selectivity: *Cydia pomonella* Granulovirus (CpGV) is highly specific and only has an effect on very few species of the Tortricidae family (Lepidoptera).
- There are no major deviations from the GAP uses previously assessed in the DAR (2008) with the exception of a slightly higher max. total rate per crop/season.
- As can be seen from the initial DAR (2008), risk quotients (Margin-of-Safety-values) clearly exceeded the default trigger values.
- Literature search submitted for the renewal of the approval for CpGV did not indicate any adverse effects on aquatic organisms associated with the use of baculoviruses (see Anonymous, 2016, BVL no 3306490; data point KMA 8/01).

Nevertheless, a quantitative risk assessment for aquatic organisms is provided below for illustrative purposes.

Effects on aquatic organisms

Effects of the formulation VIRGO on aquatic organisms have been assessed for the first submission. Therefore, all relevant data were assessed in the EU review. Risk assessments for VIRGO with the proposed use pattern are provided here and are considered adequate with regard to the evaluation of effects on aquatic organisms of the formulated product.

The toxicity of VIRGO to *Oncorhynchus mykiss*, *Daphnia magna* and *Pseudokirchneriella subcapitata* was evaluated (please refer to the OECD Dossier, Doc IIIM, Section 6, Point IIIM 10.2 and EFSA Journal 2012;10(4):2655[6]).

All available data for aquatic organisms demonstrate that CpGV as any other baculovirus and the formulated product VIRGO are not toxic, not pathogenic or infective to these organisms. Water is not the natural habitat of CpGV, therefore survival of disseminated CpGV will decrease with time. In addition, no growth and multiplication in water is expected. Nevertheless, a quantitative risk assessment confirming the safe use is provided.

The EU agreed endpoints are summarised in the following table.

Table B.9.2-4: Summary of the studies on effects for aquatic organisms

Test item	Test species	Endpoint	Reference
Fish			
CARPOVIRUSINE (1.0×10^{13} GV/L)	*Danio rerio*	96-hour (static) LC_{50} > 250 mg /L LC_{50} > 1.0×10^9 GV/L	EFSA Journal 2012;10(4):2655[7]

[6] European Food Safety Authority; Conclusion on the peer review of the pesticide risk assessment of the active substance *Cydia pomonella* granulovirus. EFSA Journal 2012;10(4):2655

[7] European Food Safety Authority; Conclusion on the peer review of the pesticide risk assessment of the active substance *Cydia pomonella* granulovirus. EFSA Journal 2012;10(4):2655

GRANUPOM (as Granulosevirus CpGV SC; 2.2 × *10[13] GV/L*)	*Oncorhynchus mykiss*	96-hour (static) LC50 > 100 mg /L LC50 > 2.0 × 109 GV/L	OECD Dossier, Doc M, IIIM, Section 6, Point IIIM 10.2 & EFSA Journal 2012;10(4):2655[7]
VIRGO (2.0 × 10^{13} GV/L)	*Oncorhynchus mykiss*	**96-hour (static)** **LC_{50} > 100 mg /L** **LC_{50} > 1.61 × 10^9 GV/L**	EFSA Journal 2012;10(4):2655[7]
Aquatic invertebrates			
CARPOVIRUSINE (1.0 × 10^{13} GV/L)	*Daphnia magna*	48-hour (static) EC_{50} > 250 mg/L EC_{50} > 1.0 × 10^9 GV/L	EFSA Journal 2012;10(4):2655[7]
GRANUPOM (as Granulosevirus CpGV SC; 2.2 × *10[13] GV/L*)	*Daphnia magna*	48-hour (static) EC50 > 100 mg/L EC50 > 2.0 × 109 GV/L	OECD Dossier, Doc M, IIIM, Section 6, Point IIIM 10.2 & EFSA Journal 2012;10(4):2655[7]
VIRGO (2.0 × 10^{13} GV/L)	*Daphnia magna*	**48-hour (static)** **EC_{50} > 100 mg/L** **EC_{50} > 1.61 × 10^9 GV/L**	EFSA Journal 2012;10(4):2655[7]
Single cell algae			
CARPOVIRUSINE (1.0 × 10^{13} GV/L)	*Pseudokirchneriella subcapitata*	72-hour (static) EC_{50} > 100 mg/L EC_{50} > 1.0 × 10^9 GV/L	EFSA Journal 2012;10(4):2655[7]
GRANUPOM (as Granulosevirus CpGV SC; 2.2 × *10[13] GV/L*)	*Scenedesmus subspicatus*	72-hour (static) EC50 > 100 mg/L EC50 > 2.0 × 109 GV/L	OECD Dossier, Doc M, IIIM, Section 6, Point IIIM 10.2 & EFSA Journal 2012;10(4):2655[7]
VIRGO (2.0 × 10^{13} GV/L)	*Pseudokirchneriella subcapitata*	**72-hour (static)** **EC_{50} > 100 mg/L** **EC_{50} > 1.61 × 10^9 GV/L**	EFSA Journal 2012;10(4):2655[7]

Endpoints used for the risk assessment are marked in **bold**

Predicted environmental density in natural waters

The envisaged field of use as a foliar treatment in may result in contamination of adjacent surface waters by spray drift. Depending on the intended use drift values for sideward application are considered. The following calculation is based on worst-case scenarios of complete accumulation of test item following 6 applications in one representative crop scenario for sideward (pome fruits and walnut).

$$\mathrm{PED_{sw}} = \frac{\text{amount reaching the water}}{\text{water volume}}$$

Where:
Amount reaching the water = accumulated application rate [mg product/m² or GV/m²] × Drift rate [%]
Water volume (30 cm water layer) = 300 L/m²

The resulting values are presented in the following table.

Table B.9.2-5: Calculation of the predicted environmental density of VIRGO and CpGV in lentic water bodies (PEDsw) after 6 applications at 0.75 L product/ha

	Application rate [a)]	**Relevant drift rate [%][b)]**	**Amount reaching the water**	**Water volume (30 cm water layer)**	**Initial PED_{SW}**
VIRGO	4.95 kg product/ha	9.21	45.59 mg/m²	300 L/m²	152 µg/L
Cydia pomonella Granulovirus (CpGV)	3.00 × 10^{13} GV/ha	9.21	8.29 × 10^8 GV/m²	300 L/m²	2.76 × 10^6 GV/L

a) Accumulated application rate, assuming no degradation between applications; calculated with a density of VIRGO of 1.1 g/cm^3
b) Drift value for 6 applications in fruit crops (late)

The maximum PED_{SW} of 2.76×10^6 GV/L (corresponding to 152 µg product/L) is used for the risk assessments resulting from the application in orchards (pome fruits and walnut) with 6 × 0.75 L product/ha.

Risk Assessment
Aquatic organisms may be exposed to CpGV entering surface waters via spray drift. The exposure calculation was based on a worst-case scenario following 6 applications at 0.75 L product/ha (corresponding to 1.5×10^{13} GV/ha) in pome fruits and walnut (orchards), assuming no degradation between the applications. This results in a PED_{sw} of 2.76×10^6 GV/L.
The risk of *Cydia pomonella* Granulovirus (CpGV) to aquatic organisms was assessed from margin of safety (MOS; corresponding to TER) values according to the following equation:

$$\mathrm{MOS} = \frac{\mathrm{EC_{50}\ [GV/L]}}{\mathrm{PED_{SW}\ [GV/L]}}$$

Based on the available data the MOS values of fish, *Daphnia* and algae for CpGV was calculated as follows.

Table B.9.2-6: Margin of safety for aquatic organisms exposed to CpGV

Use pattern	Test organism	PED_{SW} a)	Endpoint	MOS
9.0×10^{13} GV/ha in orchards	*Oncorhynchus mykiss*	2.76×10^6 GV/L	> 1.61×10^9 CFU/L	583
	Daphnia magna		> 1.61×10^9 CFU/L	583
	Pseudokirchneriella subcapitata		> 1.61×10^9 CFU/L	583

a) Based on drift from accumulated applications, assuming no degradation between applications

Based on the submitted data on effects on aquatic organisms and the intended use in fields and glasshouses, the calculated margin of safety values are high and it is anticipated that the potential risk posed to *Cydia pomonella* Granulovirus (CpGV) to fish, *Daphnia* and algae is low and acceptable.

Comments by the RMS (2020):

RMS agrees with the risk assessment provided by the notifier. From the MOS-calculations presented above, a low risk for aquatic organisms can be concluded, especially as no lethal, sublethal or pathogenic effects have been observed at the highest doses tested.

B.9.3 Effects on Bees

VIRGO is a biological insecticide formulated as suspension concentrate, containing 2×10^{13} infective granules of *Cydia pomonella* Granulovirus (CpGV) in 1 L product. The CpGV isolate contained in VIRGO is the Mexican strain (CpGV-M) which acts against larvae of the codling moth, *Cydia pomonella* in pome fruits and walnut.

VIRGO was one of the representative formulations for first approval of the active substance Cydia pomonella Granulovirus (CpGV) and also submitted now for the renewal of approval.

B.9.3.1 Toxicity to Bees

No new studies with the representative formulation VIRGO were submitted by the applicant. Therefore, this document presents a brief study summary of the already evaluated study from the initial evaluation of VIRGO (2012).

Report:	B 9.3.1/1 Colli, M. (2005): Side effects (acute oral and contact toxicity) of VIRGO on the Honey bee, *Apis mellifera* L., in laboratory (limit test). Report No.: BT008/05. Biotecnologie BT Srl, Parco Tecnologico Agroalimente dell'Umbria, Todi, Italia. BVL no 1300695
Guidelines:	OECD Guidelines for the testing of chemicals 213 (Honeybees, Acute Oral Toxicity Test) and 214 (Honeybees, Acute Contact Toxicity Test) (OECD/OCDE 1998) Guideline on test methods for evaluating the side-effects of plant protection products on honey bees, Bulletin OEPP/EPPO Bulletin 22, 203-215 (1992), No. 170
GLP:	No (pilot study in advance of GLP inspection)

Executive Summary

The test was performed as a limit test at a dose of 100 µg product/bee for both oral and contact testing. Each treatment and the control included 5 replications containing 10 bees each. In the oral route the test solution was offered in approximately 20 µL of a 50 % aqueous sucrose solution per bee. The bees starved for 2 hours before feeding. In the contact route the test substance was applied in droplets of 1 µL deionised water per bee. The toxic standard was applied at a single dose of 0.1 µg as/bee for both oral and contact testing. Observations for mortality and behavioural abnormalities took place after 24, 48 and 72 hours after treatment.

RESULTS AND DISCUSSION

Oral and contact toxicity test:

Mortality after 72 hours was 10 % in the oral toxicity test and 8.3 % in the contact toxicity test with the test substance. No behavioural abnormalities occurred in the test substance treatments and in the control. The oral and contact 72h-LD_{50} is above 100 µg VIRGO/bee after 72 hours. The toxic standard caused mortality of 98% in oral testing and 94% in contact testing.

Table B.9.3-1: Summary of mortality of the honey bees in the oral and contact toxicity test

Dosage	Mortality 3 h (%)	Mortality 24 h (%)	Mortality 48 h (%)	Mortality 72 h (%)
Oral toxicity test				
Test Item 100µg/bee	0.00	4.00	10.00	10.00
Toxic Standard 0.1 µg a.s./bee	28.57	85.71	97.96	97.96
Untreated control	0.00	0.00	0.00	0.00
Contact toxicity test				
Test Item 100µg/bee		8.33	8.33	8.33
Toxic Standard 0.1 µg a.s./bee		14.58	93.75	93.75
Untreated control		0.00	0.00	0.00

Conclusion by the applicant

The oral and contact LD_{50} is above 100 µg VIRGO/bee after 72 hours. The toxic standard caused mortality of 98% in oral testing and 94% in contact testing.

Conclusions by the RMS (2019)
RMS concludes the validity criteria of OECD Guideline 213 and 214 are met:

- less than 10% mortality in the control (oral toxicity test: 0% during the 72h test period; contact toxicity test: 0% mortality during the 72h test period)
- only a single concentration of the reference item was tested, so that a calculation of the LD_{50} for the oral and contact test were missing; the reference item showed a high mortality at the tested concentration so that the deviation has no effect on the study

Consequently, the study is considered to be acceptable and suitable for the use in risk assessment.

B.9.3.2 Infectiveness to Bees

No tests regarding the infectiveness of VIRGO were submitted. However, information on data already evaluated in the initial evaluation of *Cydia pomonella* Granulovirus (2012) are discussed in Volume 3 MA, B.9.3.2.

B.9.3.3 Pathogenicity to Bees

No tests regarding the pathogenicity of VIRGO were submitted. However, information on data already evaluated in the initial evaluation of *Cydia pomonella* Granulovirus (2012) are discussed in Volume 3 MA, B.9.3.3.

B.9.3.4 Summary and risk assessment for Bees

No new GLP studies on the toxicity, infectiveness, or pathogenicity of VIRGO to honey bees, bumble bees and solitary bees have been submitted since the first EU evaluation.
A summary of available data was presented in Table B.9.3-2.
No relevant data were submitted regarding chronic toxicity to adult honey bees, residues in pollen and nectar, and solitary bees.

Table B.9.3-2: Ecotoxicological endpoints for bees

Test item	**Test species** **Study design** **Guideline** **GLP status**	**Endpoint**	**Findings**	**Status of evaluation**	**Reference (Report No.)** **Annex point**
Carpovirusine	*Apis mellifera* (individual) Laboratory acute toxicity OECD 213/214 GLP	LD_{50} oral 48 h	> 108.4µg product/bee** (> 1.63 x 10^6 CpGV/bee)	Already evaluated	Schmitzer, S. (2006) 26194035 BVL no 3689722
		LD_{50} contact 48 h	> 100µg product/bee** (> 1.63 x 10^6 CpGV/bee)		MP B 9.3.1/1
Virgo	*Apis mellifera* (individual) Laboratory acute toxicity	LD_{50} oral 72 h	> 100 µg product/bee** (> 1.63 x 10^6 CpGV/bee)	Already evaluated	Colli, M. (2005) Rep. No.: BT008/05 BVL no 1300695

	OECD 213/214, EPPO 170 Non-GLP				
	Apis mellifera (individual) Laboratory acute toxicity OECD 213/214, EPPO 170 Non-GLP	LD_{50} contact 72 h	> 100 µg product/bee** (> 1.63×10^6 CpGV/bee)		MP B 9.3.1/1
Madex*	*Apis mellifera* (individual) Laboratory acute toxicity EPPO 170 GLP	LD_{50} oral 48 h	> 3.5×10^7 CpGV/bee**	Already evaluated	Kling, A. (2002) 20011323/01-BLEU BVL no 1914013
	Apis mellifera (individual) Laboratory acute toxicity EPPO 170 GLP	LD_{50} contact 48 h	> 4.4×10^7 CpGV/bee**		MP B 9.3.1/1

CpGV: *Cydia pomonella* Granulovirus
* tested as Granupom (also for approval of Madex Twin a comparable formulation of MADEX). The two formulations Granupom (2.2×10^{13} granules/L) and Madex/Madex Twin (3×10^{13} granules/L) contains nearly the same amount of granules/L. Therefore their comparability is considered as sufficient

Higher tier studies on honey bees
No higher tier studies on the toxicity of the active substance, nor the representative product, have been submitted.

Exposure
The recommended use pattern for VIRGO includes application in orchards (pome and stone fruits) and walnuts (0.75L product/ha). VIRGO contains a minimum of 2×10^{13} *Cydia pomonella* Granulovirus CpGV/L, and one application will be 0.656 L product/ha per LWA (leaf wall area).

Bees may be exposed to VIRGO by direct spraying while they are foraging on flowers and weeds, through contact with fresh or dried residues or by oral uptake of contaminated pollen, nectar and honey dew.

Hazard quotients
Calculations of a hazard quotient (HQ) for risk assessment of microorganisms are not suitable, therefore no calculation was made.

Risk assessment
No data on the risk assessment of solitary bees were submitted. Therefore no risk assessment on solitary bees can be carried out.

Due to the results of acute laboratory test VIRGO is considered to be virtually non-toxic to honey bees. As the calculation of a hazard quotients are not suitable for of microorganisms, no calculation was made. To investigate the infectivness and pathogenicity of *Cydia pomonella* Granulovirus (CpGV) several laboratory studies have been generated by a literature research and were evaluated (MA B.9.3.2 and B.9.3.3). These findings indicates that baculoviruses, including CpGV, are highly host specific as cross-transmission is rarely successful and infectivity is restricted to members of the genus or in some cases to the family of the original host. No toxic or phathogenic effects were obvered.

Bumble bee colonies show no adversely effects on mortality or reproduction when exposed to the used application dosages of *Cydia pomonella* Granulovirus (Mommaerts, V. et al., 2009, BVL no 3306491; MA B.9.3.1/1).

Therefore, a risk to honey bees and bumble bees resulting of the use of VIRGO is negligible.

Conclusions by the RMS (2019)
Based on the total set of data, it can be concluded that VIRGO has to be classified as non-hazardous.

B.9.4 Effects on arthropods other than bees

The following information, was already submitted in the DAR (2008) Volume 3, Annex B-9 Point 9.6 and is now summarised in more detail.

B.9.4.1 Toxicity, infectiveness and pathogenicity in arthropods other than bees

Plant protection product

Reference:	Colli, M. (2005b): Effects of VIRGO on the Aphid Parasitoid, *Aphidius rhopalosiphi* De Stefani Perez (Hymenoptera, Braconidae in Laboratory); unpublished report no. BT005/05, BVL no 1300704
Guideline:	Barrett K.L., Grandy N., Harrison, E.G., Hassan S. and Oomen P. (eds.) (1994): Guidance Document on regulatory testing procedures for pesticides with non-target arthropods. From the ESCORT I workshop (European standard characteristics of beneficial regulato-ry testing); Wageningen, The Netherlands, 28 - 30 March 1993
	Candolfi M.P., Barrett K.L., Campbell P.J., Forster R., Grandy N., Huet M-C, Lewis G., Oomen P.A., Schmuck R. and Vogt H. (eds) (2001): Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target ar-thropods. From the ESCORT II workshop (European standard characteristics of benefi-cial regulatory testing); Wageningen, The Netherlands, 21 - 23 March 2000
	Mead-Briggs M.A., Brown K., Candolfi M.P., Coulson M.J.M., Miles M., Moll M., Nienstedt K., Schuld M., Ufer A. and Mc Indoe E: (2000): A laboratory test for evaluat-ing the effects of plant protection products on the parasitic wasp, Aphidius rhopalosiphi (De Stefani Perez) (Hymenopter, Braconidae), pp. 13-25. In: Candolfi M.P., B lumel S., Forster R., Bakker F.M., Grimm C., Hassan S:A:, Heimbach U., Mead-Briggs M.A., Reber B., Schmuck R., Vogt H. (eds.) (2000): IOBC/WPRS Guidelines to evaluate side-effects of plant protection products to non-target arthropods, Dreier-Druck, Reinheim, Germany
GLP:	yes

Material and methods:

Test substance:	VIRGO; purity: 2×10^{13} GV/L
Test species:	*Aphidius rhopalosiph*; adults
Number of test animals:	5 per treatment group / 10 individuals (5 males and 5 females) per unit
Treatments:	- Control (deionised water) - 1725 mL VIRGO/ha - toxic standard
Duration:	48 hours (exposure) followed by 11 days of post-parasitation period
Test conditions:	Temperature: 20 - 21.33°C (exposure) / 20 - 24°C (post exposure); Rel. Humidity: 72 - 90%; Photoperiod: 16 h light / 8 h darkness; Light intensity: 1500 lux (exposure) / 3000 lux (post exposure)
Deviations from guideline:	None.
Endpoint:	Survival and reproduction
Observations:	Mortality after 1, 2, 24, 48 hours after introduction; reproduction rate: the mean number of offspring per female was determined for the treated and control groups by averaging the replicate values.

Results:

The mean mortality of *Aphidius rhopalosiphi* after 48 h exposure to VIRGO treated glass plates was 18% compared to 10% mortality in the control group (Table 9.4-1). The corrected mortality of VIRGO after 48 hours was calculated as 8.89%. In the reference group 100% mortality was recorded. The re-production rate of the control organisms resulted in 6.93 mummies per female. In the VIRGO treated group 8.13 mummies were produced (Table 9.4-1). The reduction in beneficial capacity of VIRGO on *Aphidius rhopalosiphi* was calculated as – 6.6%, indicating an increased beneficial capacity.

Table B.9.4-1: Mortality and reproduction rate of *Aphidius rhopalosiphi*

	Control	VIRGO 1725 mL/ha	Toxic standard
Mean mortality [%] after 48 h	10	18	100
Corrected mortality [%] after 48 h	-	8.89	-
Reduction of beneficial capacity [%]	-	-6.6*	-

* negative value: increased beneficial capacity

A summary of endpoints is given in the table below.

Toxic effects / Infectivity / Pathogenicity of plant protection product to arthropods other than bees

Test species	*Aphidius rhopalosiphi*
Toxicity of plant protection product	No signs of toxicity/infectivity/pathogenicity
	LR_{50} >1725 mL/ha

Comments by the RMS (2019):

The study is formally valid, however, since the way of infection starts with the oral intake of virus granules by larvae, dissolving in alkaline milieu of the mid gut and releasing virions, this study with treated glass plates is not applicable to assess possible effects of CpGV on *Aphidius rhopalosiphi*.
The LR_{50} was calculated to be >1725 mL/ha.

Reference:	Colli, M. (2005c): Effects of Virgo on the predatory mite, *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae) in the Laboratory (Limit Test); unpublished report

	no. BT010/05, BVL no 1300703
Guideline:	Barrett K.L., GRANDY N., Harrison, E.G., Hassan S. and Oomen P. (eds.) (1994): Guidance Document on regulatory testing procedures for pesticides with non-target arthropods. From the ESCORT I workshop (European standard characteristics of beneficial regulatory testing); Wageningen, The Netherlands, 28 - 30 March 1993 Candolfi M.P., Barrett K.L., Campbell P.J., Forster R., Grandy N., Huet M-C, Lewis G., Oomen P.A., Schmuck R. and Vogt H. (eds) (2001): Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods. From the ESCORT II workshop (European standard characteristics of beneficial regulatory testing); Wageningen, The Netherlands, 21 - 23 March 2000
GLP:	yes
Material and methods:	
Test substance:	VIRGO; purity: 2×10^{13} GV/L
Test species:	*Typhlodromus pyri*; protonymphs
Number of test animals:	- 4 per treatment group / 20 individuals per unit (mortality) - 5 pert treatment group / 5 females + 1 male per unit (reproduction)
Treatments:	- control (deionised water) - test item VIRGO at 1725 mL/ha - toxic standard ARAGOL L40 (content: 380 g Dimethoate/kg) at 0.14 g a.s./ha.
Duration:	7 days of exposure to treated glass plates, followed by a 7-day of fertility test of the surviving test organisms
Test conditions:	Temperature: 25 - 25.67°C; Rel. Humidity: 44.5 - 84.5%; Photoperiod: 17 h light / 7 h darkness; Light intensity: 1500 lux
Deviations from guideline:	Relative humidity < 60% (minimum 44.5%) instead of 75 ± 15%
Endpoint:	Survival and fecundity
Observations:	Mortality after 1, 3, 7 days after introduction; reproduction rate 14 days after exposure: the mean number of offspring per female was determined for the treated and control groups by averaging the replicate values.

Results:

The mean mortality of *Typhlodromus pyri* after 7 d of exposure to VIRGO treated glass plates was 13.75% compared to 15% mortality in the control group. In the toxic reference group 82.5% mortality was recorded.
The reproduction rate of the control organisms resulted in 4.4 eggs per female. In the VIRGO treated group 4.48 eggs per female were produced.

A summary of endpoints is given in the table below.

Toxic effects / Infectivity / Pathogenicity of plant protection product to arthropods other than bees

Test species	*Typhlodromus pyri*
Toxicity of plant protection product	No signs of toxicity/infectivity/pathogenicity
	LR_{50} >1725 mL/ha

Comments by the RMS (2019):

The study is formally valid, however, since the way of infection starts with the oral intake of virus granules by larvae, dissolving in alkaline milieu of the mid gut and releasing virions, this study with treated glass plates is not applicable to assess possible effect of CpGV on *Typhlodromus pyri*.

The LR_{50} was calculated to be >1725 mL/ha.

B.9.4.2 Risk assessment for arthropods other than bees

In RMS' point of view, no quantitative risk assessment is deemed necessary given the lack of toxicity, infectivity or pathogenicity from laboratory data in conjunction with the following available information:

- High selectivity: *Cydia pomonella* Granulovirus (CpGV) is highly specific and only has an effect on very few species of the Tortricidae family (Lepidoptera).
- There are no major deviations from the GAP uses previously assessed in the DAR (2008) with the exception of a slightly higher max. total rate per crop/season.
- As can be seen from the initial DAR (2008), risk quotients (Margin-of-Safety-values) clearly exceeded the default trigger values.
- Literature search submitted for the renewal of the approval for CpGV did not indicate any adverse effects on non-target arthropods associated with the use of baculoviruses (see Anonymous, 2016, 2016, BVL no 3306490; data point KMA 8/01).

Nevertheless, a quantitative risk assessment for arthropods other than bees is provided below for illustrative purposes.

Effects on arthropods other than bees
Effects of the formulation VIRGO on non-target arthropods other than bees have been assessed for the first submission. Therefore, all relevant data were assessed in the EU review. Risk assessments for VIRGO with the proposed use pattern are provided here and are considered adequate with regard to the evaluation of effects on non-target arthropods other than bees of the formulated product.
The toxicity of VIRGO to non-target arthropods other than bees was evaluated in laboratory tests (please refer to the OECD Dossier, Doc IIIM, Section 6, Point IIIM 10.4 and EFSA Journal 2012;10(4):2655[8]). All available data for demonstrate that CpGV as any other baculovirus and the formulated product VIRGO are not toxic, not pathogenic or infective to non-target arthropods. Nevertheless, a quantitative risk assessment confirming the safe use is provided.
The EU agreed endpoints are summarised in the following table.

Table B.9.4-2: Summary of the studies on effects to non-target arthropods

Test substance	Species	Exposed life stage	Study type	Endpoint	Reference
CARPOVIRUSINE (1.0×10^{13} GV/L)	*Hippodamia convergens*	Adult	30-day diet test	EC_{50} > 5500 ppm (5.5×10^{10} GV/g diet)	EFSA Journal 2012;10(4):2655[8]
	Chrysoperla carnea	Larvae	10-day diet test	EC_{50} > 5500 ppm (5.5×10^{10} GV/g diet)	
	Aphidius rhopalosiphi	Adult	Extended laboratory (barley seedlings)	EC_{50} > 3.0 L product/ha	
	Typhlodromus pyri	Protonymphs	Extended laboratory (bean leafs)	EC_{50} > 3.0 L product/ha	
GRANUPOM (as Granulosevirus CpGV SC; 2.2×10^{13} GV/L)	*Aphidius rhopalosiphi*	Adult	Laboratory	EC_{50} > 0.36 L product/ha (7.92×10^{12} GV/ha)	OECD Dossier, Doc M, IIIM, Sec. 6, Point 10.4 & EFSA Journal 2012;10(4):2655[8]
	Typhlodromus pyri	Protonymphs	Laboratory	EC_{50} > 0.36 L product/ha (7.92×10^{12} GV/ha)	

[8] European Food Safety Authority; Conclusion on the peer review of the pesticide risk assessment of the active substance *Cydia pomonella* granulovirus. EFSA Journal 2012;10(4):2655

	Poecilus cupreus	Adult	Extended laboratory	EC_{50} > 0.45 L product/ha (9.9×10^{12} GV/ha)	
VIRGO (2.0×10^{13} GV/L)	*Aphidius rhopalosiphi*	Adult	Laboratory	EC_{50} > 1.725 L product/ha (3.45×10^{13} GV/ha)	EFSA Journal 2012;10(4):2655[8]
	Typhlodromus pyri	Protonymphs	Laboratory	EC_{50} > 1.725 L product/ha (3.45×10^{13} GV/ha)	
Further information	Data from the literature were submitted covering laboratory studies, field trials, short and long term experiments and investigation concerning the selectivity of CpGV or related species. No harmful effects on non-target arthropods are reported. The host specifity is high. CpGV acts highly specific to Tortricidae				EFSA Journal 2012;10(4):2655[8]

Endpoints used for risk assessment are marked in **bold**

Risk assessment for arthropods other than bees

The calculation of HQ values as used for chemicals (application rate/LD_{50}) is generally regarded as less feasible for risk assessments with mBCAs because dose-response relationships are rarely observed in cases of pathogenic effects (OECD 2012[9]).

The risk of *Cydia pomonella* Granulovirus (CpGV) to non-target arthropods other than bees was assessed from margin of safety (MOS; corresponding to TER) values according to the following equation:

$$\text{MOS} = \frac{\text{EC}_{50}\ [\text{GV/ha}]}{\text{application rate}\ [\text{GV/ha}]}$$

The resulting values for the single application rates and for the accumulated application rate in pome fruits and walnut are presented in the following tables.

Table B.9.4-3: MOS calculation for the single application rate of VIRGO

Crop	**EC_{50} [GV/ha]**	**Single application rate [GV/ha]**	**MOS**
Pome fruits, walnut	> 3.45×10^{13}	1.50×10^{13}	2.30

MOS = Margin of safety

Table B.9.4-4: MOS calculation for the accumulated application rate of VIRGO

Crop	**EC_{50} [GV/ha]**	**Maximum application rate [GV/ha]**	**MOS**
Pome fruits, walnut	> 3.45×10^{13}	9.00×10^{13}	0.383

MOS = Margin of safety

A low margin of safety is derived for the exposure to non-target arthropods after the use of VIRGO after multiple applications according to GAP based on up to 6 applications. The application rate is summed in this calculation. It is very unlikely that the same population of non-target arthropods is exposed to each application. Furthermore, it is extremely worst-case to assume a cumulative application rate as the both active microorganism and the product will not be stable on the crop due to environmental conditions.

[9] OECD Guidance to the Environmental Safety Evaluation of Microbial Biocontrol Agents, Series on Pesticides No. 67, ENV/JM/MONO(2012)1

According to the Commission Regulation (EU) No 546/2011, Part II, Uniform principles for evaluation and authorisation of plant protection products containing micro-organisms[10], Part B, article 2.8.4.1, a micro-organism may give rise to risks because of its potential to infect and multiply in arthropods other than bees. Whether or not identified risks could be changed due to the formulation of the plant protection product shall be assessed, taking into account the following information on the micro-organism:

(a) its mode of action,
(b) other biological properties,
(c) studies on toxicity, pathogenicity and infectivity to honeybees and other arthropods.

And in article 2.8.4.2[10], a plant protection product may give rise to toxic effects due to the action of toxins or co-formulants. For the assessment of such effects the following information shall be taken into consideration:

(a) studies on toxicity to arthropods;
(b) information on fate and behaviour in the various parts of the environment;
(c) available data from biological primary screening.

If mortality or signs of intoxication are observed in the tests the evaluation must include a calculation of toxicity/exposure ratios based on the quotient of the ER 50 value (effective rate) and the estimated exposure.

Also in the Commission Regulation (EU) No 546/2011, Part II, Uniform principles for evaluation and authorisation of plant protection products containing micro-organisms[11], Part C, article 2.8.4., where there is a possibility of arthropods other than bees being exposed, no authorisation shall be granted if:
(a) the micro-organism is pathogenic to arthropods other than bees,
(b) in case of toxic effects due to components in the plant protection product such as relevant metabolites/toxins, unless it is clearly established through an appropriate risk assessment that under field conditions there is no unacceptable impact on those organisms after use of the plant protection product in accordance with the proposed conditions of use. Any claims for selectivity and proposals for use in integrated pest management systems shall be substantiated by appropriate data.
The tested concentration in the effect studies is clearly below the accumulated application rate used as worst case exposure scenario. However, it has to be kept in mind that no adverse effects were observed in the studies and therefore, the obtained margins of safety likely overestimate a possible risk for non-target arthropods by far. Literature information further demonstrates absence of infectivity, pathogenicity or toxicity of CpGV or any other baculovirus to arthropods other than the well-known host species within the genera *Cydia* and *Grapholita*.

Effects of *Cydia pomonella* Granulovirus on Lepidoptera species in off-crop habitats
Cydia pomonella Granulovirus (CpGV) is restricted in its infectivity to very few hosts of the Tortricidae family only. The host range of CpGV is well described. For more details please refer to Doc M-MA, Section 2, Point MA 2.3. Lepidoptera in off-crop habitats that are not hosts of CpGV will not be at risk due to application of CpGV in orchards. Therefore, no further risk assessment is provided.

Comments by the RMS (2020):

RMS agrees with the risk assessment provided by the notifier. Based on the quantitative risk assessment in conjunction with existing literature information a low risk can be concluded for non-target arthropods other than bees.

[10] Commission Regulation (EU) No 546/2011: Uniform Principles for Evaluation and Authorisation of Plant Protection Products, as provided for in Article 29(6) of Regulation (EC) No 1107/2009
[11] Commission Regulation (EU) No 546/2011: Uniform Principles for Evaluation and Authorisation of Plant Protection Products, as provided for in Article 29(6) of Regulation (EC) No 1107/2009

B.9.4.3 Effects on earthworms

The following information was already submitted in the DAR (2008) Volume 3, Annex B-9, Point 9.7 and is now summarised in more detail.

B.9.4.4 Toxicity, infectiveness and pathogenicity in earthworms

Plant protection product

Reference:	Colli, M. (2005d): Acute toxicity of VIRGO on Earthworms, *Eisenia foetida*, using an artifical soil (Limit test); unpublished report no. BT013/05, BVL no 1300705
Guideline:	OECD Guideline No. 207
GLP:	yes
Material and methods:	
Test substance:	VIRGO; purity: 2×10^{13} GV/L
Test species:	*Eisenia foetida*
Number of test animals:	10 per replicate, 50 individuals per treatment group
Treatments:	- negative control with water, - positive control (2-chloroacetamide), - test item concentration of 1000 mg/kg (limit-test)
Duration:	14 days of exposure
Test conditions:	Soil substrate: 10% sphagnum peat, 20% kaolinite clay, 70% fine sand; Food: 5g (coffee grounds and milled carrots); Temperature: 22°C; Photoperiod: Continuous light; Light intensity: 469 - 800 lux
Deviations from guideline:	None.
Endpoint:	Survival and worm body weight
Observations:	Mortality was assessed on Days 7 and 14. After identifying the surviving earthworms in each group, they were replaced on the same test substrate surface. The wet weight of surviving earthworms was assessed 14 days after test initiation.

Results:

After 14 days of exposure, 2% mortality was recorded at the test substance concentration and 6% mortality in the control group. No test item related behavioural abnormalities occurred after exposure. The average body weight increase of the surviving test organisms after 2 weeks in the VIRGO group was –0.004 g from the initial weight. In the control group the average body weight increase after 2 weeks was –0.042 g from the initial weight

A summary of endpoints is given in the table below.

Toxic effects / Infectivity / Pathogenicity of plant protection product to earthworms

Test species	*Eisenia foetida*
Toxicity of plant protection product	No signs of toxicity/infectivity/pathogenicity
	LC_{50} > 1000 mg/kg soil dw

Comments by the RMS (2019):

The study is acceptable.
The median lethal concentration LC_{50} of VIRGO to *Eisenia foetida* determined after 14 days exposure

is shown to be greater than 1000 mg/kg of artificial soil, corresponding with 1.61×10^{10} granules/kg artificial soil (assuming a density of 1.2 mg/L).

B.9.4.5 Risk assessment for earthworms

In RMS' point of view, no quantitative risk assessment is deemed necessary given the lack of toxicity, infectivity or pathogenicity from laboratory data in conjunction with the following available information:

- High selectivity: *Cydia pomonella* Granulovirus (CpGV) is highly specific and only has an effect on very few species of the Tortricidae family (Lepidoptera).
- There are no major deviations from the GAP uses previously assessed in the DAR (2008) with the exception of a slightly higher max. total rate per crop/season.
- Literature search submitted for the renewal of the approval for CpGV did not indicate any adverse effects on earthworms associated with the use of baculoviruses (see Anonymous, 2016, BVL no 3306490; data point KMA 8/01).

Nevertheless, a quantitative risk assessment for earthworms and other soil organisms is provided below for illustrative purposes.

Effects on earthworms and other soil organisms
Effects of the formulation VIRGO on earthworms have been assessed for the first submission. Therefore, all relevant data were assessed in the EU review. Risk assessments for VIRGO with the proposed use pattern are provided here and are considered adequate with regard to the evaluation of effects on earthworms of the formulated product.
The toxicity of VIRGO to earthworm was evaluated (please refer to the OECD Dossier, Doc IIIM, Section 6, Point IIIM 10.5 and EFSA Journal 2012;10(4):2655[12]).
All available data for earthworms demonstrate that CpGV as any other baculovirus and the formulated product VIRGO are not toxic, not pathogenic or infective. Nevertheless, a quantitative risk assessment confirming the safe use is provided.
The EU agreed endpoints are summarised in the following table.

Table B.9.4-5: Summary of the studies on effects to earthworms

Test substance	Test species	Endpoint	Reference
CARPOVIRUSINE (6.7×10^{12} GV/L)	*Eisenia fetida*	14-day, acute 1000 mg product/kg soil (dw)*	OECD Dossier, Doc M, IIIM, Sec. 6, Point 10.5 & EFSA Journal 2012;10(4):2655[12]
CARPOVIRUSINE (1.0×10^{13} GV/L)	*Eisenia fetida*	14-day, acute 1000 mg product/kg soil (dw)*	
	Eisenia fetida	56-day, reproduction 1000 mg product/kg soil (dw)*	
GRANUPOM (as Granulosevirus CpGV SC; 2.2×10^{13} GV/L)	*Eisenia fetida*	14-day, acute 1000 mg product/kg soil (dw) (1.67×10^{10} GV/kg soil (dw))*	EFSA Journal 2012;10(4):2655[12]
VIRGO (2.0×10^{13} GV/L)	*Eisenia fetida*	14-day, acute **1000 mg product/kg soil (dw)** **(1.61×10^{10} GV/kg soil (dw))***	EFSA Journal 2012;10(4):2655[12]

***** No signs of infectivity or pathogenicity to earthworms have been observed
Endpoints used for the risk assessment are marked in **bold**

Predicted environmental population density in soil
In order to perform a risk assessment for non-target organisms the actual population of *Cydia pomonella*

[12] European Food Safety Authority; Conclusion on the peer review of the pesticide risk assessment of the active substance *Cydia pomonella* granulovirus. EFSA Journal 2012;10(4):2655

Granulovirus (CpGV) is calculated for soil, based on the maximum accumulated application rate of 4.5 L product/ha in pome fruits and walnut upon foliar application, assuming 6 treatments of 0.75 L/ha and as a worst case no degradation between the multiple applications. The resultant amount of active substance will be related to the top 5 cm of soil to achieve the highest theoretical soil population.
For the calculation the content of 2.0×10^{13} GV/L product has been considered.

Assumptions:

- Application rate VIRGO: 0.75 L product/ha (equivalent to 1.5×10^{13} GV/ha)
- Accumulated application rate (up to 6 treatments): 4.5 L product/ha, equivalent to 9.0×10^{13} GV/ha
- Incorporation into the top 5 cm layer (resulting soil volume V = 0.05 m × 10,000 m² = 500 m³)
- Soil density ρ of 1.5 g/cm³ (= 1.5×10^3 kg/ m³)
- Soil mass / ha: V × ρ = 750,000 kg soil dry weight
- Plant interception is not considered in the calculation as it is generally assumed that this parameter is not applicable for microbial pest control agents and products.

The actual density of viable spores of *CpGV* in soil (PED_{soil}) considering the worst-case scenario is calculated as

$$PED_{soil} = \frac{\text{accumulated application rate}}{(V \times \rho)}$$

Where:
Accumulated application rate in [GV/ha] or [kg product/ha]
Soil volume V = 500 m³
Soil density ρ = 1.5×10^3 kg/ m³

The resulting values are presented in the following table.

Table B.9.4-6: Calculation of the predicted environmental density of VIRGO and CpGV in soil (PED_{soil}) after 6 applications at 0.75 L product/ha

Accumulated application rate [kg product/ha]*	**Rate [mg product/m²]***	**Soil depth [cm]**	**Bulk density [g/cm³]**	**Initial PED related to soil depth [mg product/kg soil (dw)]***
4.95	495	5.00	1.5	6.60
Accumulated application rate [GV/ha]	**Rate [GV/m²]**	**Soil depth [cm]**	**Bulk density [g/cm³]**	**Initial PED related to soil depth [GV/kg soil (dw)]**
9.0×10^{13}	9.0×10^9	5.00	1.5	1.20×10^8

* calculated with a density of VIRGO of 1.1 g/cm³

According to the PED_{soil} calculation the expected initial density is 6.60 mg product/kg dry soil, corresponding to 1.20×10^8 GV/kg dry soil.

Risk Assessment
The acute toxicity of VIRGO to *Eisenia fetida* has been investigated a 14-day acute laboratory studies. The LC_{50} was determined to be above 1000 mg product/kg soil (dw) (corresponding to 1.61×10^{10} GV/kg soil (dw)). No signs of clinical toxicity or abnormal behaviour were observed.
Long-term exposure of earthworms and long-term risks with respect to e.g. reproduction are considered unlikely.
A worst-case scenario was chosen that assumes complete accumulation following 6 applications at 0.75 L product/ha in pome fruits and walnut. The predicted environmental density in soil (PED_{soil}) was calculated as 1.20×10^8 GV/kg soil dw (corresponding to 6.60 mg product/kg soil dw) for multiple application in pome fruits and walnut, assuming a worst case scenario that no interception and no degradation occurs between applications.

The risk of *Cydia pomonella* Granulovirus (CpGV) to earthworms was assessed from margin of safety (MOS, corresponding to TER) values according to the following equation:

$$\text{MOS} = \frac{\text{LC50[mg product/kg soil dw]}}{\text{PEDsoil [mg product/kg soil dw]}}$$

Based on the available data the MOS values of earthworm exposure to CpGV was calculated as follows.

Table B.9.4-7: MOS calculation for earthworms

Use pattern	Test organism	LC_{50} [mg product/kg soil (dw)]	PED_{soil} [mg product/kg soil (dw)]	MOS
6 × 0.75 L product/ha in pome fruits and walnut	*Eisenia fetida*	1.61×10^{10}	1.20×10^{8}	134.2

MOS = Margin of safety

The calculated MOS value is high, indicating an acceptable acute risk to earthworms after application of VIRGO at the maximum recommended use rate. Literature information further demonstrates absence of infectivity, pathogenicity or toxicity of CpGV or any other baculovirus to earthworms.

Comments by the RMS (2020):

RMS agrees with the risk assessment provided by the applicant. Based on the quantitative risk assessment a low risk can be concluded for earthworms.

B.9.5 Effects on non-target soil micro-organisms

The following information was already submitted in the DAR (2008) Volume 3, Annex B-9, Point 9.8 and is now summarised in more detail.

Reference:	Ragni, A. (2005): Assessment of the effects of VIRGO on soil respiration and nitrification; unpublished report no. BT009/05, BVL no 1300710
Guideline:	OECD Guideline 216 and 217
GLP:	Yes
Material and methods:	
Test substance:	VIRGO; purity: 2×10^{13} GV/L
Reference substance:	Dinoseb acetate
Treatments:	Negative control soil was not treated. Virgo: 3.0×10^{13} and 10.0×10^{13} GV/ha (equivalent to 1.5 and 5.0 L/ha) Dinoseb acetate: 25 mg/kg as positive control
Duration:	28 days
Test conditions:	Agricultural soil from permanent grassland (sandy soil). Temperature: 20°C ± 2°C; Photoperiod: darkness
Deviations from guideline:	None.
Endpoint:	Nitrogen turnover, short-term respiration
Observations:	The measurements were performed on days 0, 7, 14 and 28 of the incubation period. Nitrate concentration

	Nitrate concentration was determined in three replicates per group. The nitrate content was determined using Ion Chromatography technique. Glucose induced respiration Samples were mixed with a sufficient amount of glucose to elicit an immediate maximum respiratory response. The respiration rate was assessed from the oxygen consumed by the glucose amended soil samples for 12 consecutive hours, by the use of OxiTop System ®. The results were expressed as mg oxygen/kg dry weight/h.

Results:

The validation of the nitrates extracting method is submitted in KIII, Section 6, Point 10.6/02, Ragni (2005; BMF2006-19, BVL no 1300709).
In all treatment groups, the deviation of the nitrate content of the sandy soil was less than 25% from the control group within the 28 days incubation period. In particular at day 28 the deviation value from the control soil was 1.8% and 0.4%, respectively for the low and high doses of the test substance. Therefore, the impact on soil nitrogen turnover is considered as negligible even at 5.0 L/ha of VIRGO.
The short term respiration of the soil microflora was not significantly different from the control over a 28 d period after admixture with glucose at 1.5 L/ha and 5.0 L/ha dosage. The deviation from the control was distinctly below the limit of 25%.
The effect of toxic reference substance on short-term respiration and on nitrogen turnover in soil was studied in a parallel trial to assess the soil microflora sensitivity (Dottorini, 2005b; unpublished report no. BT027/05, BVL no 1300707). The toxic standard Dinoseb acetate had a clear inhibition effect on the soil microflora in the short-term respiration and in the nitrogen turnover (deviation from the control > 25%) after 28 days of incubation.

Comments by the RMS (2019):

The study is acceptable.
The impact of VIRGO on respiration and microbial nitrification of sandy soil is considered as negligible (< 25% deviation) even at 7.5 L/ha VIRGO, corresponding with 15×10^{13} granules/ha.

Reference:	Dottorini, F. (2005): Assesment of the effects of VIRGO (CpGV $2x10^{13}$ GV/lt, SC) on soil repiration and nitrification; unpublished report no. BT028/05, BVL no 1300708
Guideline:	OECD Guideline 216 and 217
GLP:	Yes
Material and methods:	
Test substance:	VIRGO; purity: 2×10^{13} GV/L
Reference substance:	The effect of toxic reference substance on short-term respiration and on nitrogen turnover in soil was studied in a parallel trial by Dottorini (2005b; unpublished report no. BT027/05, BVL no 1300707. 2005c; unpublished report no. BT024/05, BVL no 1300706) to assess the soil microflora.
Treatments:	Negative control soil was not treated. VIRGO: 4.5×10^{13} and 15.0×10^{13} GV/ha (equivalent to 2.25 and 7.5 L/ha)
Duration:	28 days
Test conditions:	Agricultural soil from a non-cultivated field (sandy soil). Temperature: 20°C ± 2°C; Photoperiod: darkness
Deviations from guideline:	None.
Endpoint:	Nitrogen turnover, short-term respiration
Observations:	The measurements were performed on days0, 7, 14 and 28 of the incubation period.

Nitrate concentration

Nitrate concentration was determined in three replicates per group. The nitrate content was determined using Ion Chromatography technique.

Glucose induced respiration

Samples were mixed with a sufficient amount of glucose to elicit an immediate maximum respiratory response. The respiration rate was assessed from the oxygen consumed by the glucose amended soil samples for 12 consecutive hours, by the use of OxiTop System ®. The results were expressed as mg oxygen/kg dry weight/h.

Results:

During the incubation time the CO_2 produced by the soil treated with the low and high dose of test substance was in the range from –13.5% to 0.0% in comparison to the control soil.
The content of nitrate in soil, treated with low and high dose of test substance was in the range from –9.8% to 2.9% in comparison with the untreated control soil.
The toxic standard Dinoseb acetate had a clear inhibition effect on the soil microflora in the short-term respiration and in the nitrogen turnover (deviation from the control > 25%) after 28 days of incubation.

Comments by the RMS (2019):

The study is acceptable.
The impact of VIRGO on respiration and microbial nitrification of sandy soil is considered as negligible (< 25% deviation) even at 7.5 L/ha VIRGO, corresponding with 15×10^{13} granules/ha.

B.9.5.1 Impact on non-target soil micro-organisms

No detrimental impacts on non-target soil micro-organisms with regard to functional endpoints were noted.

B.9.5.2 Risk assessment for non-target soil micro-organisms

In RMS' point of view, no quantitative risk assessment is deemed necessary given the lack of toxicity, infectivity or pathogenicity from laboratory data in conjunction with the following available information:

- High selectivity: *Cydia pomonella* Granulovirus (CpGV) is highly specific and only has an effect on very few species of the Tortricidae family (Lepidoptera).
- There are no major deviations from the GAP uses previously assessed in the DAR (2008) with the exception of a slightly higher max. total rate per crop/season.
- Literature search submitted for the renewal of the approval for CpGV did not indicate any adverse effects on non-target soil micro-organisms associated with the use of baculoviruses (see Anonymous, BVL no 3306490, 2016; data point KMA 8/01).

Nevertheless, a quantitative risk assessment for soil micro-organisms is provided below for illustrative purposes.

Effects on soil micro-organisms

Effects of the formulation VIRGO on soil micro-organisms have been assessed for the first submission. Therefore, all relevant data were assessed in the EU review. Risk assessments for VIRGO with the proposed use pattern are provided here and are considered adequate with regard to the evaluation of effects on soil micro-organisms of the formulated product
The toxicity of VIRGO to soil micro-organisms was evaluated (please refer to the OECD Dossier, Doc

IIIM, Section 6, Point IIIM 10.6 and EFSA Journal 2012;10(4):2655[13]).
All available data demonstrate that CpGV as any other baculovirus and the formulated product VIRGO are does not have any effect on soil microorganisms.
The EU agreed endpoints are summarised in the following table.

Table B.9.5-1: Summary of the studies on effects to soil micro-organisms

Test substance	Test design	Endpoint	Reference
CARPOVIRUSINE (1.0×10^{13} GV/L)	C	2.7×10^{7} GV/kg soil (dw) (corresponding to 2.0×10^{13} GV/ha)	OECD Dossier, Doc M, IIIM, Sec. 6, Point 10.6 & EFSA Journal 2012;10(4):2655[13]
	N		
GRANUPOM (2.2×10^{13} GV/L)	C	1.33×10^{8} GV/kg soil (dw) (corresponding to 1.0×10^{14} GV/ha)	EFSA Journal 2012;10(4):2655[13]
	N		
VIRGO (2.0×10^{13} GV/L)	C	1.33×10^{8} GV/kg soil (dw) (corresponding to 1.0×10^{14} GV/ha)	EFSA Journal 2012;10(4):2655[13]
	N		
VIRGO (2.0×10^{13} GV/L)	C	**2.0×10^{8} GV/kg soil (dw) (corresponding to 1.5×10^{14} GV/ha)**	EFSA Journal 2012;10(4):2655[13]
	N		

C: carbon transformation, N: nitrogen turnover
Endpoints used for the risk assessment are marked in **bold**

Risk assessment
The toxicity of VIRGO against soil micro-organisms has been investigated in two laboratory studies over 28 days. The impact on nitrogen transformation and soil respiration in studies was considered as negligible (< 25% deviation) after 28 days.
A worst-case scenario was chosen that assumes complete accumulation following 6 applications at 0.75 L product/ha in pome fruits and walnut. The predicted environmental density in soil (PED_{soil}) was calculated as 1.20×10^{8} GV/kg soil dw (corresponding to 1.548 mg product/kg soil dw) for multiple application in pome fruits and walnut, assuming a worst-case scenario that no interception and no degradation occurs between applications.

Table B.9.5-2: Risk assessment for soil micro-organisms

Use pattern	Test organism	PED_{soil} [GV/kg soil (dw)]	Endpoint [GV/kg soil (dw)]
6 × 0.75 L product/ha in pome fruits and walnut	Soil microorganism	1.20×10^{8}	2.00×10^{8}

Cydia pomonella Granulovirus (CpGV) had no significant effect on soil functional parameters nitrogen conversion and carbon transformation at 2.00×10^{8} GV/kg soil (dw), corresponding to 1.5×10^{14} GV/ha. Due to the absence of adverse effects observed in the laboratory study with VIRGO, it can be assumed that GAP directed use of VIRGO poses no risk for the soil microflora responsible for nitrogen conversion and carbon transformation. Literature information further demonstrates absence of infectivity, pathogenicity or toxicity of CpGV or any other baculovirus to soil microorganisms.

[13] European Food Safety Authority; Conclusion on the peer review of the pesticide risk assessment of the active substance *Cydia pomonella* granulovirus. EFSA Journal 2012;10(4):2655

Comments by the RMS (2020):

RMS agrees with the risk assessment provided by the applicant. Based on the quantitative risk assessment a low risk can be concluded for soil-microorganisms.

B.9.6 Additional studies

No additional studies have been conducted with VIRGO.

B.9.7 References relied on

Data point	Author(s)	Year	Title Owner, Report No. Source (where different from owner) GLP or GEP status Published or not 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
KMA 8/01	Anonymous	2016	LITERATURE REVIEW REPORT ON CYDIA POMONELLA GRANULOVIRUS - EFFECTS ON NON-TARGET ORGANISMS Arysta LifeScience S.A.S., not applicable not available GLP/GEP: no Published: no 3306490	no	yes	New data for active ingredient, not previously submitted nor evaluated	ALS	N
KMA 8.3	Mommaerts, V., Sterk, G., Hoffmann, L., Smagghe, G.	2009	A LABORATORY EVALUATION TO DETERMINE THE COMPATIBILITY OF MICROBIOLOGICAL CONTROL AGENTS WITH THE POLLINATOR BOMBUS TERRESTRIS 59632 Pest management science N/N J 3306491	no	no		LIT	
KMP 10.2	[redacted]	2005	ACUTE TOXICITY TESTING OF VIRGO IN RAINBOW TROUT (ONCORHYNCHUS MYKISS) (TELEOSTEI, SALMONIDAE) Sipcam S.p.A., 20051166/01-AAOm [redacted] GLP: yes Published: no 3964532	yes	no	not protected	SIP	Y KIIIM 10.2
KMP 10.2	Fifi, A.P.	2005a	EVALUATION OF VIRGO (CPGV 2X10^{13} GV/LT, SC) TOXIC EFFECTS ON DAPHNIA MAGNA USING TEST OF ACUTE IMMOBILISATION	no	no	not protected	SIP	Y KIIIM 10.2

			Sipcam S.p.A., BT007/05 Biotecnologie BT Srl, Fraz. Pantalla, Italy GLP: yes Published: no 3964533					
KMP 10.2	Fifi, A.P.	2005b	DETERMINATION OF VIRGO (CPGV 2X10^{13} GV/LT, SC) TOXIC EFFECTS ON THE ALGAL GROWTH (PSEUDOKIRCHNERIELLA SUBCAPITATA) Sipcam S.p.A., BT006/05 Biotecnologie BT Srl, Fraz. Pantalla, Italy GLP: yes Published: no 3964534	no	no	not protected	SIP	Y KIIIM 10.2
KMP 10.2	Fifi, A.P.	2005c	TOXIC EFFECTS OF VIRGO (CPGV 2X1013 GV/LT, SC) ON THE DUCKWEED GROWTH (LEMNA MINOR). Sipcam S.p.A., BT012/05 Biotecnologie BT Srl, Fraz. Pantalla, Italy GLP: yes Published: no 3964535	no	no	not protected	SIP	Y KIIIM 10.2
KMP 10.3	Schmitzer, S.	2006	EFFECTS OF CARPOVIRUSINE (ACUTE CONTACT AND ORAL) ON HONEY BEES (APIS MELLIFERA L.) IN THE LABORATORY Arysta LifeScience S.A.S., 26194035 Institut für Analytik u. Umweltchemie GmbH, Germany GLP: yes Published: no 3689722	no	no	not protected	ALS	Y KIIIM 10.3
KMP 10.3	Colli, M.	2005	SIDE EFFECTS (ACUTE ORAL AND CONTACT TOXICITY) OF VIRGO ON THE HONEY BEE, APIS MELLIFERA L., IN LABORATORY (LIMIT TEST). Sipcam S.p.A., BT008/05 Biotecnologie BT Srl, Fraz. Pantalla, Italy GLP: yes Published: no 3964542/ BIE2006-68	no	no	not protected	SIP	Y KIII M 10.3

KMP 10.3	Kling, A.	2002	ASSESSMENT OF SIDE EFFECTS OF GRANUPOM TO THE HONEY BEE, APIS MELLIFERA L. IN THE LABORATORY Andermatt Biocontrol GmbH / Probis GmbH, 20011323/01-BLEU ArGe GAB Biotech/IFU, Niefern-Öschelbronn, Germany GLP: yes Published: no 3687401	no	no	not protected	PKA	Y KIII M 10.3
KMP 10.4	Colli, M.	2005b	EFFECTS OF VIRGO ON THE APHID PARASITOID, APHIDIUS RHOPALOSIPHI DE STEFANI PEREZ (HYMENOPTERA, BRACONIDAE IN LABORATORY) Sipcam S.p.A., BT005/05 Biotecnologie BT Srl, Fraz. Pantalla, Italy GLP: yes Published: no 3964544	no	no	not protected	SIP	Y KIIIM 10.4
KMP 10.4	Colli, M.	2005c	EFFECTS OF VIRGO ON THE PREDATORY MITE, TYPHLODROMUS PYRI SCHEUTEN (ACARI, PHYTOSEIIDAE) IN THE LABORATORY (LIMIT TEST) Sipcam S.p.A., BT010/05 Biotecnologie BT Srl, Fraz. Pantalla, Italy GLP: yes Published: no 3964545	no	no	not protected	SIP	Y KIIIM 10.4
KMP 10.5	Colli, M.	2005d	ACUTE TOXICITY OF VIRGO ON EARTHWORMS, EISENIA FOETIDA, USING AN ARTIFICAL SOIL (LIMIT TEST) Sipcam S.p.A., BT013/05 Biotecnologie BT Srl, Fraz. Pantalla, Italy GLP: yes Published: no 3964565	no	no	not protected	SIP	Y KIIIM 10.5
KMP 10.6	Ragni, A.	2005a	ASSESSMENT OF THE EFFECTS OF VIRGO ON SOIL RESPIRATION AND NITRIFICATION Sipcam S.p.A., BT009/05 Biotecnologie BT Srl, Fraz. Pantalla, Italy	no	no	not protected	SIP	Y KIIIM 10.6

			GLP: yes Published: no 3964567					
KMP 10.6	Ragni, A.	2005b	VALIDATION OF THE NITRATES EXTRACTING METHOD FROM AGRICULTURAL SOIL MATRIX USING IC TO QUANTIFY THEIR CONTENT Sipcam S.p.A., BT002/05 Biotecnologie BT Srl, Fraz. Pantalla, Italy GLP: yes Published: no 3964568	no	no	not protected	SIP	Y KIIIM 10.6
KMP 10.6	Dottorini, F.	2005a	ASSESSMENT OF THE EFFECTS OF VIRGO (CPGV 2 X 1013 GRANULES/LT, SC) ON SOIL RESPIRATION AND NITRIFICATION Sipcam S.p.A., BT028/05 Biotecnologie BT Srl, Fraz. Pantalla, Italy GLP: yes Published: no 3964569	no	no	not protected	SIP	Y KIIIM 10.6
KMP 10.6	Dottorini, F.	2005b	ASSESSMENT OF THE INHIBITION EFFECTS OF DINOSEB ACETATE ON SOIL RESPIRATION AND NITRIFICATION. Sipcam S.p.A., BT027/05 Biotecnologie BT Srl, Fraz. Pantalla, Italy GLP: yes Published: no 3964570	no	no	not protected	SIP	Y KIIIM 10.6
KMP 10.6	Dottorini, F.	2005c	NITRATES EXTRACTING METHOD FROM AGRI-CULTURAL SOIL MATRIX USING IC (IONIC CHROMATOGRAPHY) TO QUANTIFY THEIR CONTENT - VALIDATION Sipcam S.p.A., BT024/05 Biotecnologie BT Srl, Fraz. Pantalla, Italy GLP: yes Published: no 3964571	no	no	not protected	SIP	Y KIIIM 10.6