Renewal Assessment Report

Cydia pomonella GV

Madex

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

Rev. 0 – 16 October 2020

Rapporteur Member State: Germany

Co-Rapporteur Member State: The Netherlands

Version history

When	What
16 October 2020	First version submitted to EFSA

The RMS is the author of the Assessment Report. The Assessment Report is based on the validation by the RMS, and the verification during the EFSA peer-review process, of the information submitted by the Applicant in the dossier, including the Applicant's assessments provided in the summary dossier. As a consequence, data and information including assessments and conclusions, validated and verified by the RMS experts, may be taken from the applicant's (summary) dossier and included as such or adapted/modified by the RMS in the Assessment Report. For reasons of efficiency, the Assessment Report should include the information validated/verified by the RMS, without detailing which elements have been taken or modified from the Applicant's assessment. As the Applicant's summary dossier is published, the experts, interested parties, and the public may compare both documents for getting details on which elements of the Applicant's dossier have been validated/verified and which ones have been modified by the RMS.

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B Summary, evaluation and assessment of the data and information

B.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 MADEX (*Cydia pomonella* Granulovirus (CpGV)).

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

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

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) a) per use b) per crop/ season	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 fruit Stone fruit Walnut	F	Codling moth (*Cydia pomonella*)	Foliar spray (tractor drawn)	Before first larvae hatch from eggs*	a) 10 (6-8**) b) 10 (6-8**)	a) 0.3 × 10^{13} GV/ha b) 3 × 10^{13} GV/ha	a) 0.1 b) 1	400 / 1200
Pome fruit Stone fruit Walnut	HG ***		Foliar spray (Knapsack sprayer)					

mch = m crown height
*First treatment 85 day degrees after the first warm evening with flight activity. Zero point of development of the codling moth is 10°C.
** 6-8 sunny days, counting 2 partially sunny days as 1 day
*** HG: Home garden use

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 and is now summarised in more detail.

In general, it is referred to the information submitted for the active substance. The substances of the preparations Granupom or MADEX 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 Toxicity, invectiveness and pathogenicity in birds

Plant protection product

No data submitted.

B.9.1.2 Risk assessment for birds

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 MADEX 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 MADEX are inert and no hazards to birds and mammals are expected (please refer to Doc J (ABA)). 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 MADEX 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

[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

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. Therefore, 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[7]. 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.9.1-9 (chapter on aquatic organisms).

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:

$$\text{MOS} = \frac{\text{LD50 [GV/kg bw]}}{\text{daily dose [GV/kg bw]}}$$

[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.].
[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

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 MADEX 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
Small insectivorous bird - tit, wren	0.010	0.002697	7.5×10^9	1.5×10^9	4.05×10^8	$> 1.0 \times 10^{11}$	> 247
Small herbivorous mammal - vole	0.025	0.003579			2.15×10^8	$> 4.9 \times 10^{10}$	> 228

[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 MADEX.

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, Points 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]. (1998a): Acute toxicity testing of granulosevirus CpGV SC in rainbow trout (*Oncorhynchus mykiss*) (Teleostei, Salmonidae); unpublished report no. 96272/01-AAOm, BVL no 3687395
Guideline:	OECD Guideline 203 and Annex to commission Directive 92/69/F-EC, procedure C.1
GLP:	Yes
Material and methods:	
Test substance:	Granulosevirus CpGV SC; purity: 2.2×10^{13} granula/L
Test species:	Rainbow trout (*Oncorhynchus mykiss*); weight: 1 - 2 g; length: 4.0 - 6.0 cm

[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

Number of test animals:	Control group: 10, Treated groups: 10
Treatments:	0, 0.01, 0.1, 1.0, 10 and 100 mg/L
Duration:	96 hours
Test conditions:	Static system; Temperature: 14.0 - 16.9°C; Photoperiod: 12 - 16 hour photoperiod; Oxygen content: > 60% of the air saturation value; Hardness: Approx. 20°dH; pH: 7.2 - 7.8; no feeding d *Oncorhynchus mykiss* uring the main test
Deviations from guideline:	- A main test was not performed as at the limit concentration of 100 m/L no significant mortality could be observed. On the base of the results a main test below 100 mg/L would not give additional information - The temperature was up to 1°C lower than outlined in the study protocol. But this cir-cumstance did not influence the validity of the test as the control group was not influ-enced in any way.
Endpoint:	Survival, subelthal effects
Observations:	Daily check for mortality, occurrence of sublethal effects (loss of equilib-rium, erratic swimming loss of reflex, excitability, discolouration, or change in behaviour), dissolved oxygen, pH and temperature

Results:

Up to a nominal concentration level of 10 mg/L, one fish died by chance at 0.1 mg/L. At this concentration level no lethal or sublethal effects were observed on the remaining nine organisms. The mortality may be caused by an attack of one of the others. The one dead fish at 100 mg/L represents no significant mortality caused by the test substance but it corresponds to the observed sublethal effects caused by the test substance as outlined below. According to these results, no main test was required because no significant mortality could be expected up to the limit concentration of 100 mg/L.
No significant mortality neither sublethal effect was observed at all concentrations below the nominal concentration level of 100 mg/L over 96 h with the exception of a single dead fish at 0.1 mg/L who died by chance. At 100 mg/L all fish showed a change in pigmentation to a dark colour. Therefore, the concentration of 100 mg/L represented the LOEC in this test.

Table B.9.2-1: Observation of clinical signs of fish during the main test

Granulo-sevirus CpGV SC [mg/L]	**Control**					**0.01**					**0.1**				
Time [h]	0	°	*	#	+	0	°	*	#	+	0	°	*	#	+
0	10				0	10				0	10				0
3	10				0	10				0	10				0
6	10				0	10				0	10				0
24	10				0	10				0	9			1	0
48	10				0	10				0	9				1
72	10				0	10				0	9				1
96	10				0	10				0	9				1
Granulo-sevirus CpGV SC [mg/L]	**1.0**					**10**					**100**				
Time [h]	0	°	*	#	+	0	°	*	#	+	0	°	*	#	+
0	10				0	10				0	10				0
3	10				0	10				0	10				0
6	10				0	10				0	10				0
24	10				0	10				0	9		1		0
48	10				0	10				0	9		1		0
72	10				0	10				0	8		1	1	0
96	10				0	10				0	8			1	1

O: no clinical signs
°: Unusual behaviour (reduced activity and /or orientation to bottom or surface of the vessel)
*: difficulties with maintenance of equilibrium
#: fish upside down with loss of equilibrium, showing only movement of gills as a sign of life
+: no sign of life

A summary of endpoints is given in the table below.

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

Test species	Rainbow trout (*Oncorhynchus mykiss*)
Toxicity of plant protection product	signs of stress in the 100 mg/L treatment group; 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. The NOEC is 10 mg/L.
The change of pigmentation was a discussion point during the PRAS M5 Expert meeting in 9 February 2012. It was noted that the test compound is brown and maybe the optical change caused stress to the fish. It is known that Rainbow trout reacts to visibility. Overall, it is considered that it is likely that the effects were due to the fish being stressed in the toxicity study rather than an indication of pathogenicity

or infectivity.

The following conclusion was made during the PRAS M5 Expert meeting in 9 February 2012:
"The experts considered that the risk assessment issues for *Cydia pomonella* GV from the proposed use of the representative products are sufficiently addressed for non-target wild species (except bees)".

B.9.2.2 Effects on freshwater invertebrates

Plant protection product

Reference:	[redacted] (1998b); Acute toxicity testing of granulosevirus CpGV SC on *Daphnia magna* using the 48 h acute immobilisation test; unpublished report no. 96272/01-AADm, BVL no 3687396
Guideline:	OECD Guideline 202, Part I: Daphnia sp., Acute Immobilisation Test and Reproduc-tion Test and Annex to Commission Directive 92/69/EEC, procedure C.2.
GLP:	Yes
Material and methods:	
Test substance:	Granulosevirus CpGV SC
Test species:	*Daphnia magna* Straus, clone 5
Number of test animals:	Control groups: 20, Treated groups: 20
Treatments:	0.1, 1.0, 10 and 100 mg/L
Duration:	48 hours
Test conditions:	Static system; Temperature: 20 ± 1°C;Photoperiod: 16 hours photoperiod daily; Oxygen content: >60% of air saturation; pH: 6.5 - 8.5; no food during the test
Deviations from guideline:	1- As no effects were expected, the concentration level of 0.01 mg/L was not performed in the range-finding test. 2- A main test was not performed as no observable effects took place during the range-finding test. These deviations were not considered to have affected the outcome or the objectives of the study.
Endpoint:	immobility
Observations:	Daily check for mortality/immobilization, dissolved oxygen, pH and temperature.

Results:

The immobilisation in the range-finding test was 0% up to 100 mg/L after 48 h of test duration.

Table 9.2-2: Mortality of *Daphnia magna*

Nominal concentration (mg/L)	Cumulative mortality (%)		
	hours	24 h	48 h
Control	0	0	0
0.1	0	0	0
1.0	0	0	0
10	0	0	0
100	0	0	0

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 EC_{50} is > 100 mg product/L, the NOEC is 100 mg product/L.

B.9.2.3 Effects on algae growth

Plant protection product

Reference:	Dengler, D. (1998): Testing of toxic effects of granulosevirus CpGV SC on the single cell green alga *Scenedesmus subspicatus*; unpublished report no. 96272/01-AASs, BVL no 3687397
Guideline:	OECD Guideline 201: Alga, Growth Inhibition Test and EEC Directive C.3, Alga inhibition test.
GLP:	Yes
Material and methods:	
Test substance:	Granulosevirus CpGV SC; purity: 2.2×10^{13} granula/L
Test species:	*Scenedesmus subspicatus* (= *Desmodesmus subspicatus*) Chodat, Strain No. 8681
Number of test animals:	n.a. (initial cell density: 10^4 algal cells per mL)
Treatments:	0, 0.01, 0.1, 11, 10 and 100 mg Granulosevirus CpGV SC/L
Duration:	72 hours
Test conditions:	static system; Temperature: 23°C ± 2°C; Photoperiod: Continuous illumination with a light intensity of approx. 8000 Lux; Test units: Erlenmeyer flasks of 500 mL volume with 200 mL test medium;pH: 8.13 to 8.28 at test start and pH 7.93 to 10.40 at test end
Deviations from guideline:	none
Endpoint:	biomass and growth rate
Observations:	daily cell density measurements and daily check for test conditions

Results:

Based on the results of the range-finding test, the main test was performed at a limit test design with 6 controls without test substance and 6 concentrations with 100 mg/L Granulosevirus CpGV SC. No inhibitory effects were detected during the 72h incubation period.

Table B.9.2-1: Results of the limit test (mean cell numbers)

Time [h]	Cells / mL * 10^{-4} #	
	Control	Granulosevirus CpgV SC
0	1.00	1.00
24	5.73	4.95
48	35.68	45.31
72	173.70	187.30

Algal counts are divided by 1000. At the start, 10000 cells were incubated

A summary of endpoints is given in the table below.

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

Test species	*Scenedesmus subspicatus* (= *Desmodesmus subspicatus*)
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 EC_{50} was estimated to be >100 mg/L. On the basis of the observations made during the test, the NOEC was determined to be > 100 mg/L.

B.9.2.4 Effects on plants other than algae

Plant protection product

Reference:	Dengler, D. (2002): Assessment of toxic effects of Granupom on aquatic plants using the duckweed *Lemna gibba*; unpublished report no. 20011323/01-AALg, BVL no 3687399
Guideline:	OECD Guidelines for the Testing of Chemicals: *Lemna* sp. Growth Inhibition Test, Proposal for a New Guideline 221, October 2000.
GLP:	Yes
Material and methods:	
Test substance:	Granupom; purity: 3.4×10^{10} granula/L
Test species:	*Lemna gibba* G3
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: 23 - 25°C; Photoperiod: Continuous illumination with a light intensity of approx. 6500 Lux; Test units: 100 mL glass beakers each filled with a volume of approx. 500 mL of test solution; pH: 7.5
Deviations from guideline:	None
Endpoint:	Frond number, frond size, final dry weight, appearance, necrosis or mortality
Observations:	Frond numbers in each test vessel were determined at the start of the test. Frond numbers and the appearance of the colonies were checked at days 3, 5 and 7 as well as any change in plant development, frond size, appearance, necrosis or mortality and additional observations of test media or other abnormalities. The mean initial dry weight of the inoculum plants per test vessel was determined by collecting 3 representative samples at test initiation. The final dry eight of the yield from each test vessel was determined at the end of the test period. Plants in the respective vessels were collected, blotted dry and dried in glass dishes at 60°C to a constant weight. Any root fragments were included. The average specific growth rate (μ) for exponentially growing cultures was calculated.

Results:

Table B.9.2-2: Mean frond numbers

Conc. [mg/L]	0 d		3 d		5 d		7 d	
	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.
0.00	12	0.0	28	2.2	98	16.4	289	29.2
100	12	0.0	29	2.3	130	13.2	320	26.6

Table B.9.2-3: Mean growth rates [l/d]

Conc. [mg/L]	3 d		5 d		7 d	
	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.
0.00	0.2796	0.0267	0.4165	0.0346	0.4536	0.0156
100	0.2971	0.0250	0.4571	0.0217	0.4684	0.0121

Table B.9.2-4: Percentage of inhibition of growth rates

Conc. [mg/L]	3 d	5 d	7 d
0.00	0.0	0.0	0.0
100	-6.3	-14.0	-3.3

Table B.9.2-5: Mean doubling time [T_d]

Conc. [mg/L]	3 d	5 d	7 d	
	T_d [days]			T_d [h]
0.00	2.479	1.664	1.528	36.7
100	2.333	1.459	1.480	35.5

Table B.9.2-6: Biomass increase per 12 fronds [µg] and % inhibition at the end of the test

Conc. [mg/L]	Biomass increase		I_b [%]
	Mean	Std. Dev.	
0	28.9	2.3	0.0
100	31.0	2.5	-7.3

Analytical results:

The concentration course of the formulation Granupom was tested in growth medium during the main test by analysing the active ingredient CpGV over the whole test period of 7 days by means of a bioassay. For this purpose the liquids of the replicates were pooled and analysed afterwards.
Granupom nominal contains 2.2×10^{10} granula per mL, and 3.4×10^{10} of virus-granula were reanalysed in the certificate of analysis. 100 mg of Granupom per L correspond to 91.7 µl/L containing nominal 2.02×10^{6} granula per mL and analysed 3.12×10^{6} granula per mL.
All initial concentrations were found within the limit of 80% of the nominal concentrations. Two of the aged solutions showed reduced values of virus concentration probably due to adhesion of granula at the glass vessel during the test.

A summary of endpoints is given in the table below.

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

Test species	*Lemna gibba*
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 test results can be regarded to be valid, as the doubling time of control frond numbers was calculated at 36.7 h. This is less than demanded in the OECD test guideline (60 h).
No LOEC and EC_{50} could be determined for any growth parameter; the NOEC can be set to be ≥ 100 mg/L.

B.9.2.5 Risk assessment for aquatic organisms

Table B.9.2-7: Summary of the studies on toxicity on aquatic organisms treated with toxin/metabolite from the active ingredient or the plant protection product Granupom (Granulosevirus CpGV SC).

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, 0.01, 0.1, 1.0, 10 and 100 mg/L	LC50 > 100 mg/L, NOEC = 10 mg/L	No signs of toxicity/pathogenicity; signs of stress in the 100 mg/L treatment group	[redacted] (1998a), BVL no 3687395
Water flea (*Daphnia magna*)	48 hours	0, 0.1, 1.0, 10 and 100 mg/L	EC50 > 100 mg/L, NOEC ≥ 100 mg/L	No signs of toxicity/pathogenicity	[redacted] (1998b), BVL no 3687396
Green algae (*Pseudokirchneriella subcapitata*)	72 hours	0, 0.01, 0.1, 1.0, 10 and 100 mg/L	EC50 > 100 mg/L, NOEC ≥ 100 mg/L	No signs of toxicity/pathogenicity	Dengler, D. (1998), BVL no 3687397
Duckweed (*Lemna gibba*)	7 days	0 and 100 mg/L	EC50 > 100 mg/L, NOEC ≥ 100 mg/L	No signs of toxicity/pathogenicity	Dengler, D. (2002), BVL no 3687399

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

No experimental data for MADEX were submitted for the first approval of *Cydia pomonella* Granulovirus (CpGV) to address the pathogenicity and infectiveness to aquatic organisms. Effects of the formulation GRANUPOM on aquatic organisms have been assessed for the first submission. GRANUPOM (or Granulosevirus CpGV SC) contains the same co-formulants as MADEX. Therefore, studies conducted with GRANUPOM (or Granulosevirus CpGV SC) are fully applicable to assess possible effects of MADEX on aquatic organisms. All relevant data were assessed in the EU review. Risk assessments for MADEX 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 GRANUPOM (or Granulosevirus CpGV SC) to *Oncorhynchus mykiss, Daphnia magna and Scenedesmus subspicatus* 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 MADEX 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.9.2-8: 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[6]
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[6]
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[6]
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[6]
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[6]
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[6]
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[6]

[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

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[6]
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[6]

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 10 applications in one representative crop scenario for sideward (pome fruits and walnut).
The predicted environmental density of CpGV in lentic water bodies (PED_{sw}) is calculated as

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

Where:
Amount reaching the water = accumulated application rate [mg product/m² or GV/m²] × Drift rate [%]
Water volume (30 cm water layer) = 300 L/m^2

The resulting values are presented in the following table.

Table B.9.2-9: Calculation of the predicted environmental density of MADEX and CpGV in lentic water bodies (PED_{sw}) after 10 applications at 0.1 L product/ha

	Application rate [a]	Relevant drift rate [%][b]	Amount reaching the water	Water volume (30 cm water layer)	Initial PED_{sw}
MADEX	1.161 kg product/ha	8.66	10.054 mg/m²	300 L/m²	33.5 µg/L
Cydia pomonella Granulovirus (CpGV)	3.00 × 10^{13} GV/ha	8.66	2.60 × 10^{8} GV/m²	300 L/m²	8.66 × 10^{5} GV/L

[a] Accumulated application rate, assuming no degradation between applications; calculated with a density of MADEX of 1.161 g/cm^3
[b] Drift value for more than 7 applications in fruit crops (late)

The maximum PED_{SW} of 8.66 × 10^{5} GV/L (corresponding to 33.5 µg product/L) is used for the risk assessments resulting from the application in orchards (pome fruits and walnut) with 10 × 0.1 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 10 applications at 0.1 L product/ha (corresponding to 3.0 × 10^{13} GV/ha) in pome fruits and walnut (orchards), assuming no degradation between the applications. This results in a PED_{sw} of 8.66 × 10^{5} 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:

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

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

Table B.9.2-10: Margin of safety calculation for aquatic organisms exposed to CpGV

Use pattern	Test organism	PED_{SW} [a)]	Endpoint	MOS
3.0×10^{13} GV/ha in orchards	*Oncorhynchus mykiss*	**8.66×10^{5} GV/L**	> 2.0×10^{9} CFU/L	2309
	Daphnia magna		> 2.0×10^{9} CFU/L	2309
	Scenedesmus subspicatus		> 2.0×10^{9} CFU/L	2309

[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 glass-houses, 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

MADEX is a biological insecticide formulated as suspension concentrate, containing 3×10^{13} infective granules of *Cydia pomonella* Granulovirus (CpGV) in 1 L product. The CpGV isolate contained in MADEX is the Mexican isolate (CpGV-M) which acts highly specific against larvae of the codling moth, *Cydia pomonella*.
MADEX was one of the representative formulations for first approval of the active substance CpGV and also submitted now for the renewal of approval.

B.9.3.1 Toxicity to Bees

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

Report:	B 9.3.1/1
	Kling, A. (2002), Assessment of Side Effects of Granupom to the Honey Bee, *Apis mellifera* L. in the Laboratory, Project n° 20011323/01-BLEU, BVL no 1914013
Guidelines:	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
Deviations:	To guarantee high food uptake of the bees in the oral toxicity test, the starvation phase was prolonged (2 hours 45 minutes instead of 2 hours).
	Observations were made under neon light instead of red light due to a better visibility of bees and their behaviour under neon light.
GLP:	Yes
Validity:	Yes

Executive Summary

The oral and contact toxicity of Granupom to the Honey bee (*Apis mellifera* L.) was determined in a limit test according to the EPPO Guideline No. 170 (EPPO, 1992). The bees were exposed to the highest possible dose of 4.4×10^7 granula per bee of Granupom by feeding and topical application. The concentration of Granupom in the feeding solution was intentionally set 25% higher than needed to achieve the nominal dosage of 4.4×10^7 granula per bee with the quantity of 250 µL offered per cage to compensate for a potential decrease in food uptake of bees frequently observed in such tests.

In the oral toxicity test the maximum nominal test lever (4.4×10^7 granula per bee) corresponded to an actual intake of 3.5×10^7 granula per bee. At this concentration a corrected mortality of 18.4% was observed after 72 hours.

At the concentration of 4.4×10^7 granula per bee (pure product) which was tested in the contact toxicity test with Granupom no mortality (corrected mortality: -4.2%) occurred after 48 hours.

In the control of the oral toxicity test a mortality of 2.0% was observed after 72 hours. A mortality of 4.0% occurred in the control of the contact toxicity test after the 48 hours observation period.

Regarding the behaviour, the treated bees did not differ from the control at any time during the test.

According to the results of this study it can be assumed that the oral LD_{50}/72 h is above 3.5×10^7 granula per bee and the contact LD_{50}/48 h of Granupom is above 4.4×10^7 granula per bee.

RESULTS AND DISCUSSION

Oral toxicity test:

The nominal test concentration of 4.4×10^7 granula per bee corresponded to an actual intake of 3.5×10^7 granula per bee. At this concentration the corrected mortality was determined to be 18.4% after 72 hours. 2.0% mortality was observed in the control group after 72 hours. Regarding the behaviour, the treated bees did not differ from the control at any time during the test.

Table B.9.3-1: Corrected average mortality in the oral toxicity test with Granupom as a function of the intake of test substance, the toxic standard and the control

Treatment	Intake of test substance [µg a.s./bee]	Mortality [%]			Mortality [%] (corrected for control)		
		24 h	48 h	72 h	24 h	48 h	72 h
Control	--	2.0	2.0	2.0	-	-	-
Test substance: Granupom							
4.4×10^7 granula/bee	3.5×10^7	4.0	20.0	20.0	2.0	18.4	18.4
Toxic standard: "Perfekthion"							
0.15 µg a.s./bee	0.18	94.0	96.0	96.0	93.9	95.9	95.9

Contact toxicity test:

At the concentration of 4.4×10^7 granula per bee which was tested in the contact toxicity test with Granupom no mortality (corrected mortality: -4.2%) was observed after 48 hours. In the control group a mean mortality of 4.0% occurred after 48 hours. Regarding the behaviour, the treated bees did not differ from the control at any time during the test.

Table B.9.3-2: Corrected average mortality in the contact toxicity test as a function of the concentration of test substance applied to the thorax of the bees

Treatment	Mortality [%]		Mortality [%] (corrected for control)	
	24 h	48 h	24 h	48 h
Control	4.0	4.0	-	-
Test substance: Granupom				
4.4×10^7 granula/bee	0.0	0.0	-4.2	-4.2
Toxic standard: "Perfekthion"				
0.21 µg a.s./bee	72.0	84.0	70.8	83.3

Conclusions by the applicant

According to the results of this study it can be assumed that the oral LD_{50}/72 h of Granupom is above 3.5×10^7 granula per bee and the contact LD_{50}/48 h is above 4.4×10^7 granula per bee. Regarding the behaviour, the treated bees did not differ from the control at any time during the test.

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: 2% during the 72h test period; contact toxicity test: 4% 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 MADEX 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 MADEX 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 MADEX to honey bees, bumble bees and solitary bees have been submitted since the first EU evaluation.
A summary of available data is presented in Table 9.3-3.
No relevant data were submitted regarding chronic toxicity to adult honey bees, residues in pollen and nectar, and solitary bees.

Table B.9.3-3: 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 OECD 213/214, EPPO 170 Non-GLP	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
	Apis mellifera (individual)	LD_{50} contact 72 h	> 100 µg product/bee** (> 1.63 x 10^6 CpGV/bee)		MP B 9.3.1/1

	Laboratory acute toxicity OECD 213/214, EPPO 170 Non-GLP				
Madex*	*Apis mellifera* (individual) Laboratory acute toxicity EPPO 170 GLP	LD_{50} oral 48 h	> 3.5 x 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 x 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 x 10^{13} granules/L) and Madex/Madex Twin (3 x 10^{13} granules/L) contains nearly the same amount of ganules/L. Therefore their comparability is considered as sufficient
** EU agreed endpoint; EFSA Journal 2012; 10 (4):2655

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 MADEX includes application in orchards (pome fruits) and walnuts (0.1L product/ha). MADEX contains a minimum of 3x10^{13} *Cydia pomonella* Granulovirus CpGV/L, and one application will be 0.875 L product/ha per LWA (leaf wall area).

Bees may be exposed to MADEX 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 MADEX 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 MADEX is negligible.

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

B.9.3.5 Effects on arthropods other than bees

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

B.9.3.6 Toxicity, infectiveness and pathogenicity in arthropods other than bees

Plant protection product

Reference:	Kühner, C. (2001): Granulosevirus CpGV SC: Acute toxicity to the aphid parasitoid, *Aphidius rhopalosiphi* (Hymenoptera, Braconidae) in the laboratory; unpublished report no. 96272/01-NLAp, BVL no 1914014
Guideline:	POLGÁR (1988), MEAD-BRIGGS (1992) and Guidance Document for Regulatory Testing Procedures for Pesticide with Non-Target Arthropods (ESCORT 1994)
GLP:	Yes
Material and methods:	
Test substance:	Granulosevirus CpGV SC; purity: 2.2 × 1013/L
Test species:	*Aphidius rhopalosiphi* (Hymenoptera, Braconidae); adult
Number of test animals:	4 replicates of 10 individuals (5 males and 5 females) per group
Treatments:	0 and 360 mL/ha; toxic standard: 0.85 mL Perfekthion/ha (37.4% dimethoate)
Duration:	48 h exposure of adults to treated glass plates, followed by 12 days of fertility test of the female adults
Test conditions:	Temperature: 20 ± 3°C; Rel. Humidity: 50 - 85%; Photoperiod: Long day conditions (16 h light/8 h darkness); approx. 1000 lux
Deviations from guideline:	None
Endpoint:	Survival and reproduction
Observations:	Mortality of the adults was evaluated after approx. 30min, 2h, 24h and 48h. Counting of the parasitized aphids was carried out 12 days after the start of the fertility test.

Results:

Mortality:
After 48 h, in each treated group and the control group, 1 adult was dead. The mortality of *Aphidius rhopalosiphi* was calculated as 2.5% in each group.
The corrected mortality (M) of *Aphidius rhopalosiphi* after exposure to Granulosevirus CpGV SC was calculated as 0%. In the toxic standard no adults survived.

Fecundity:
In the control group 18 females were tested in the fertility test. The total number of mummies developed within 11 days was 237 for the control group which corresponds to 13.2 mummies per female.
In the Granulosevirus CpGV SC group 19 females were tested, which produced 222 mummies. The number of mummies per female was calculated as 11.7.
The reduction in reproduction rate after exposure to Granulosevirus CpGV SC was calculated as 11.4%.
The average mortality of the test organisms after 48 h exposure to Granulosevirus CpGV SC was 2.5% compared to 2.5% mortality in the control group. The corrected mortality (M) was calculated as 0%.
The number of mummies per female during the fertility test was 11.7 in the Granulosevirus CpGV SC group compared to 13.2 mummies per female in the control. The combination of the corrected pre-imaginal mortality (M) of the test organisms with the factor r resulted in a reduction of beneficial capacity of 11.3%.

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} >360 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 >360 mL/ha.

Reference:	Kühner, C. (1998): Granulosevirus CpGV SC: Acute toxicity to the predatory mite, *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae) in the laboratory; unpublished report no. 96272/01-NLTp, BVL no 1914015
Guideline:	Louis/Ufer (1995) and Guidance Document for Regulatory Testing Procedures for Pesticide with Non-Target Arthropods (ESCORT 1994)
GLP:	Yes
Material and methods:	
Test substance:	Granulosevirus CpGV SC; purity: 2.2×10^{13}/L
Test species:	*Typhlodromus pyri;* protonymphs (1 day old)
Number of test animals:	5 replicates containing 20 mites for each variant
Treatments:	0 and 360 mL/ha; toxic standard: 100 mL Perfekthion/ha (37.4% dimethoate)
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 ± 3°C; Rel. Humidity: 70 ± 15%%; Photoperiod: Long day conditions (16 h light/8 h darkness)

Deviations from guideline:	None
Endpoint:	Survival and fecundity
Observations:	Mortality of the mites was evaluated on day 3 and 7.

Results:

Mortality:
Seven days after treatment, 85 mites were found alive in the group treated with Granulosevirus CpGV SC; 11 mites were missing and 4 mites were dead. The surviving adults were determined as 34 females and 51 males. The mortality was calculated as 15% based on the number of dead and missing mites.
In the control group 89 mites were found alive, 10 mites were missing and 1 mite was dead. The surviving adults were determined as 42 females and 47 males. The mortality was calculated as 11% based on the number of dead and missing mites. The corrected mortality for Granulosevirus CpGV SC was calculated as 4.5%.
In the toxic standard group treated with dimethoate, no surviving adults were found.

Fecundity:
During the 7 day egg laying period the number of offspring per female during the fecundity test was 5.0 in the control and in the Granulosevirus CpGV SC treated group, respectively. The factor of reproduction for the Granulosevirus CpGV SC group was calculated as 1.00.

Reduction of beneficial capacity:
The reduction in beneficial capacity in the Granulosevirus CpGV SC treated group was determined as 4.5%.

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} >360 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 *Typhlodromus pyri*.
The LR_{50} was calculated to be >360 mL/ha.

Reference:	Kühner, C. (1997): Granulosevirus CpGV SC: Acute toxicity to the ground beetle, *Poecilus cupreus* L. (Coleoptera, Carabidae) in the laboratory; unbpublished report no. 96272801-NLPc, BVL no 1914016
Guideline:	BBA Guideline VI 23-2.1.8 (Heimbach 1991) and Guidance Document for Regulatory Testing Procedures for Pesticides with Non-Target Arthropods (ESCORT 1994)
GLP:	Yes
Material and methods:	
Test substance:	Granulosevirus CpGV SC; purity: 2.2×10^{13}/L
Test species:	*Poecilus cupreus* L.; 6-9 week old beetles
Number of test animals:	5 replicates containing 3 pairs of beetles for each variant

Treatments:	0 and 360 mL/ha; toxic standard: 800 g Afugan 30 EC/ha (30.3% pyrazophos)
Duration:	14 days of exposure
Test conditions:	Beetles were exposed on moistened quartz sand. Prior to the application the beetles were introduced to the test vessels, and *Musca* pupae (2 for every beetle) were added as food. In order to simulate field application conditions the test substance solution was applied using an automatic laboratory spraying-cabin; Temperature: 20 ± 2°C (short time minimum: 16°C); Photoperiod: 16 h light : 8 h dark; Light intensity: 500 - 1000 lux; Food: Feeding with 2 *Musca* pupae per living beetle on day 1, 3, 5, 8 and 12
Deviations from guideline:	The minimum temperature was 16°C for short periods; the min/max humidity was 50/90% due to technical reasons.
Endpoint:	Survival and feeding capacity
Observations:	Mortality was recorded 2, 4 and 6 hours after application and again on day 2, 3, 5, 8, 12 and 15. Feeding capacity: the beetles in the test were fed on day 1, 3, 5, 8 and 12. At every feeding session the old pupae were removed. All pupae, eaten completely or gnawed at and all pupae, which could not be found, were recorded as eaten. At the final assessment the sand in the vessels was investigated and buried pupae were recorded.

Results:

Behaviour of the test organisms:
Observation started on the day of application. The beetles in the control and in the Granulosevirus CpGV SC treated group showed normal activity during the entire exposure period.

Mortality:
In the Granulosevirus CpGV SC group and the control group no mortality was observed.
The corrected mortality (M) for Granulosevirus CpGV SC was calculated at 0.0%.
In the toxic standard group treated with pyrazophos, a mortality of 100% was assessed.

Feeding capacity:
The average number of eaten pupae per beetle (mean value of 5 replicates) was 4.23 in the Granulosevirus CpGV SC group compared to 5.27 pupae in the control. The reduction in feeding capacity of the beetles in the Granulosevirus CpGV SC was calculated as 19.73%.
The reduction of the feeding capacity of the beetles in the toxic standard group was calculated as 93.17%.

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	*Poecilus cupreus*
Toxicity of plant protection product	No signs of toxicity/infectivity/pathogenicity
	LR_{50} >360 mL/ha

Comments by the RMS (2019):

The study is acceptable.
The LR_{50} was calculated to be >360 mL/ha.

B.9.3.7 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, 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
No experimental data for MADEX were submitted for the first approval of *Cydia pomonella* Granulovirus (CpGV) to address the pathogenicity and infectiveness to non-target arthropods other than bees. Effects of the formulation GRANUPOM on non-target arthropods other than bees have been assessed for the first submission. GRANUPOM (or Granulosevirus CpGV SC) contains the same co-formulations as MADEX. Therefore, studies conducted with GRANUPOM (or Granulosevirus CpGV SC) are fully applicable to assess possible effects of MADEX on non-target arthropods other than bees. All relevant data were assessed in the EU review. Risk assessments for MADEX 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 GRANUPOM (or Granulosevirus CpGV SC) 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[7]).

All available data for demonstrate that CpGV as any other baculovirus and the formulated product MADEX 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.9.3-4: 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[7]
	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	*Aphidius*	Adult	Laboratory	**EC_{50} > 0.36 L**	OECD Dossier,

[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

(as Granulosevirus CpGV SC; 2.2×10^{13} GV/L*)*	*rhopalosiphi*			**product/ha (7.92×10^{12} GV/ha)**	Doc M, IIIM, Sec. 6, Point 10.4 & EFSA Journal 2012;10(4):2655[7]
	Typhlodromus pyri	Protonymphs	Laboratory	**EC_{50} > 0.36 L product/ha (7.92×10^{12} GV/ha)**	
	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[7]
	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[7]

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 microbial biocontrol agents (mBCAs) because dose-response relationships are rarely observed in cases of pathogenic effects (OECD 2012[8]).

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.9.3-5: MOS calculation for the single application rate of MADEX

Crop	**EC_{50} [GV/ha]**	**Single application rate [GV/ha]**	**MOS**
Pome fruits, walnut	> 7.92×10^{12}	3.00×10^{12}	2.64

MOS = Margin of safety

Table B.9.3-6: MOS calculation for the accumulated application rate of MADEX

Crop	**EC_{50} [GV/ha]**	**Maximum application rate [GV/ha]**	**MOS**
Pome fruits, walnut	> 7.92×10^{12}	3.00×10^{13}	0.264

MOS = Margin of safety

A low margin of safety is derived for the exposure to non-target arthropods after the use of MADEX after multiple applications according to GAP based on up to 10 applications. The application rate is

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

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.
According to the Commission Regulation (EU) No 546/2011, Part II, Uniform principles for evaluation and authorisation of plant protection products containing micro-organisms[9], 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[17], 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[9], 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

[9] 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

other than bees.

B.9.4 Effects on earthworms

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

B.9.4.1 Toxicity, infectiveness and pathogenicity in earthworms

Plant protection product

Reference:	Wachter, S. (1998a): Acute toxicity of CpGV SC on earthworms, *Eisenia foetida* using an artificial soil test; unpublished report no. 96272/01-NLEf, BVL no 3687407
Guideline:	OECD Guideline No. 207
GLP:	Yes
Material and methods:	
Test substance:	CpGV SC; purity: 2.2×10^{13} /L
Test species:	*Eisenia foetida* (Michaelsen); more than 2 months-old with clitellum
Number of test animals:	10 per group
Treatments:	- negative control treated with water; - toxic standard consisting of 10, 18, 32, 56, 100 mg 2-chloroacetamide/kg artificial soil; - a geometric series of five concentrations of 100, 178, 316, 562, 1000 mg CpGV SC/kg
Duration:	14 days of exposure
Test conditions:	Soil substrate: 10% sphagnum peat, 20% kaolinite clay, 69% fine sand, Approx. 1% calcium carbonate (pH adjusted to 6.0 ± 0.5); Temperature: 20°C ± 2.0°C; Photoperiod: Continuous light; Light intensity: 400 - 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. The pH value of the substrates was controlled at the end of the test. Mean moisture of the substrate was assessed at the end of the test from 3 samples of the control group after 30-hour oven exposure at 50 ± 2°C. Biomass was evaluated in measuring the earthworms mean weights in each group.

Results:

No mortality was observed in the negative control and at all test concentrations over the test period.
The LC_{50} of 2-chloroacetamide was found to be between 18 and 32 mg/kg.
The average body weight of the test organisms in the CpGV SC group was between 79.7% and 86.4% from the initial weight. In the control group the average body weight was 82.1% from the initial weight. No significant difference between the body weights compared with the control was recorded at all test concentrations (Dunnett's t-Test).

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 CpGV SC to *Eisenia foetida* determined after 14 days exposure is shown to be greater than 1000 mg/kg of artificial soil, corresponding with 1.67×10^{10} granules/kg artificial soil (assuming a density of 1.2 mg/L).

B.9.4.2 Risk assessment for earthworms

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 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
No experimental data for MADEX were submitted for the first approval of *Cydia pomonella* Granulovirus (CpGV) to address the pathogenicity and infectiveness to earthworms. Effects of the formulation GRANUPOM on earthworms have been assessed for the first submission. GRANUPOM (or Granulosevirus CpGV SC) contains the same co-formulations as MADEX. Therefore, studies conducted with GRANUPOM (or Granulosevirus CpGV SC) are fully applicable to assess possible effects of MADEX on earthworms. All relevant data were assessed in the EU review. Risk assessments for MADEX 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 GRANUPOM (or Granulosevirus CpGV SC) 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[10]).
All available data for earthworms demonstrate that CpGV as any other baculovirus and the formulated product MADEX 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.9.4-1: Summary of the studies on effects to earthworms

Test substance	Test species	Endpoint	Reference

[10] 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

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[11]
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[11]
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[11]

* 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* Granulovirus (CpGV) is calculated for soil, based on the maximum accumulated application rate of 1.0 L product/ha in pome fruits and walnut upon foliar application, assuming 10 treatments of 0.1 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 3.0×10^{13} GV/L product has been considered.

Assumptions:

- Application rate MADEX: 0.1 L product/ha (equivalent to 3.0×10^{13} GV/ha)
- Accumulated application rate (up to 10 treatments): 1.0 L product/ha, equivalent to 3.0×10^{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.9.4-2: Calculation of the predicted environmental density of MADEX and CpGV in soil (PED_{soil}) after 10 applications at 0.1 L product/ha

Accumulated application rate [kg	Rate [mg product/m²]*	Soil depth [cm]	Bulk density [g/cm³]	Initial PED related to soil depth

[11] 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

product/ha]*				**[mg product/kg soil (dw)]***
1.161	116.1	5.00	1.5	1.548
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)]**
3.0×10^{13}	3.0×10^{9}	5.00	1.5	4.00×10^{7}

* calculated with a density of MADEX of 1.161 g/cm³

According to the PED_{soil} calculation the expected initial density is 1.548 mg product/kg dry soil, corresponding to 4.00×10^7 GV/kg dry soil.

Risk Assessment

The acute toxicity of GRANUPOM (or Granulosevirus CpGV SC) 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 1.67×10^{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 10 applications at 0.1 L product/ha in pome fruits and walnut. The predicted environmental density in soil (PED_{soil}) was calculated as 4.00×10^7 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.

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:

$$\mathrm{MOS} = \frac{\mathrm{LC50[GV/kg\ soil\ dw]}}{\mathrm{PEDsoil\ [GV/kg\ soil\ dw]}}$$

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

Table B.9.4-3: Exposure assessment for earthworms

Use pattern	**Test organism**	**LC_{50} [mg product/kg soil (dw)]**	**PED_{soil} [mg product/kg soil (dw)]**	**MOS**
10 × 0.1 L product/ha in pome fruits and walnut	*Eisenia fetida*	1.67×10^{10}	4.00×10^{7}	417.5

MOS = Margin of safety

The calculated MOS value is high, indicating an acceptable acute risk to earthworms after application of MADEX 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 notifier. 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, highlighted in grey, was already submitted in the DAR (2008) Volume 3,

Annex B-9, Point 9.8 and is now summarised in more detail.

Reference:	Wachter, S. (1998b): Assessment of the side effects of CpGV SC of the activity of the soil microflora; unpublished report no. 96272/01-ABMF, BVL no 3687408
Guideline:	BBA-Guideline for the official testing of pesticides, part VI, 1-1, 2nd edition, dated March 1990, with a sandy and a sandy loam soil type.
GLP:	Yes
Material and methods:	
Test substance:	CpGV SC; purity: 2.2×10^{13} CpGV/L
Reference substance:	Herbogil liquide (a.i. dinoterb)
Treatments:	
Duration:	
Test conditions:	Specified as sandy soil and a sandy loam soil type. Temperature: 20°C ± 2°C; Photoperiod: darkness; Moisture content: 58% of the WHC_{max}
Deviations from guideline:	None.
Study design:	Distinct fractions of dry soil were prepared by adding water so that the moisture was equivalent to 58% of the total water holding capacity. The fractions were then incubated in the dark, at 20°C ± 2°C.
Experimental treatment:	The measurements were performed within 3 hours of addition of pesti-cide, and then on days 14 and 28 of the incubation period.; Nitrate concentration: Nitrate concentration was determined in three replicates per group; Glucose induced respiration: Samples were mixed with a sufficient amount of glucose to elicit an im-mediate maximum respiratory response. The respiration rate was as-sessed from the oxygen consumed by the glucose amended soil samples for

Results:

In the treatment groups, the deviation of the nitrate contents of soil type 1 and soil type 2 was less than 15 % from the control group within the 28 days incubation period. The nitrite and ammonium content of both soil types was found to be under the determination limit. The total content of nitrogen (expressed as N_{min}) was in the range of the control group and was not significantly different from the control. Therefore, the impact on soil nitrogen turnover is considered as negligible even at 10-x dosage rate of CpGV SC.
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-x and 10-x dosage. The reference substance inhibited the short term respiration and stimulated the content of nitrogen within the 28 days incubation in the soil type 1 test. In soil type 2 the reference substance stimulated the short term respiration and the content of nitrogen within the 28 days incubation. The deviation from the control was distinctly more than 15% for both soil types.

Comments by the RMS (2019):

The study is acceptable.
The impact on nitrogen transformation and soil respiration of soil type 1 and soil type 2 is considered as negligible (< 15% deviation) even at the 10-x dosage (5.0 L/ha) of the highest recommended CpGV SC application rate, corresponding with 10×10^{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

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, 2016, BVL no 3306490; data point KMA 8/01).

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

Effects on soil micro-organisms
No data for MADEX were submitted for the first approval of *Cydia pomonella* Granulovirus (CpGV) to address the pathogenicity and infectiveness to soil micro-organisms. Effects of the formulation GRANUPOM on soil micro-organisms have been assessed for the first submission. GRANUPOM (or Granulosevirus CpGV SC) contains the same co-formulations as MADEX. Therefore, studies conducted with GRANUPOM (or Granulosevirus CpGV SC) are fully applicable to assess possible effects of MADEX on soil micro-organisms. All relevant data were assessed in the EU review. Risk assessments for MADEX 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 GRANUPOM (or Granulosevirus CpGV SC) 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[12]).

All available data demonstrate that CpGV as any other baculovirus and the formulated product MADEX 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)	EFSA Journal 2012;10(4):2655[12]
	N		
GRANUPOM (as Granulosevirus CpGV SC; 2.2×10^{13} GV/L)	C	**1.33×10^{8} GV/kg soil (dw) (corresponding to 1.0×10^{14} GV/ha)**	OECD Dossier, Doc M, IIIM, Sec. 6, Point 10.6 & EFSA Journal 2012;10(4):2655[12]
	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[12]

[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

	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[12]
	N		

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

Risk assessment

The toxicity of GRANUPOM (or Granulosevirus CpGV SC) against *soil micro-organisms* has been investigated in two soils in a laboratory study over 28 days. The impact on nitrogen transformation and soil respiration in both soil types was considered as negligible (< 25% deviation) after 28 days.
A worst-case scenario was chosen that assumes complete accumulation following 10 applications at 0.1 L product/ha in pome fruits and walnut. The predicted environmental density in soil (PED_{soil}) was calculated as 4.00×10^{7} 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: Exposure assessment for soil micro-organisms

Use pattern	Test organism	PED_{soil} [GV/kg soil (dw)]	Endpoint [GV/kg soil (dw)]
10 × 0.1 L product/ha in pome fruits and walnut	Soil microorganism	4.00×10^{7}	1.33×10^{8}

Cydia pomonella Granulovirus (CpGV) had no significant effect on soil functional parameters nitrogen conversion and carbon transformation at 1.33×10^{8} GV/kg soil (dw), corresponding to 1.0×10^{14} GV/ha. Due to the absence of adverse effects observed in the laboratory study with GRANUPOM (or Granulosevirus CpGV SC), it can be assumed that GAP directed use of MADEX 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.

Comments by the RMS (2020):

RMS agrees with the risk assessment provided by the notifier. 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 MADEX.

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 GLP/GEP: NO PUBLISHED: NO 3306491	no	no		LIT	
KMP 10.2	[redacted]	1998a	ACUTE TOXICITY TESTING OF GRANULOSEVIRUS CPGV SC IN RAINBOW TROUT (ONCORHYNCHUS MYKISS) (TELEOSTEI, SALMONIDAE) Andermatt Biocontrol GmbH / Probis GmbH, 96272/01-AAOm ArGe GAB Biotech/IFU, Niefern-Öschelbronn, Germany GLP: yes Published: no 3687395	yes	no	not protected	PKA	Y KIIIM 10.2

KMP 10.2	[redacted]	1998b	ACUTE TOXICITY TESTING OF GRANULOSEVIRUS CPGV SC ON DAPHNIA MAGNA USING THE 48 H ACUTE IMMOBILISATION TEST Andermatt Biocontrol GmbH / Probis GmbH, 96272/01-AADm ArGe GAB Biotech/IFU, Niefern-Öschelbronn, Germany GLP: yes Published: no 3687396	no	no	not protected	PKA	Y KIIIM 10.2
KMP 10.2	Dengler, D.	1998	TESTING OF TOXIC EFFECTS OF GRANULOSEVIRUS CPGV SC ON THE SINGLE CELL GREEN ALGA SCENEDESMUS SUBSPICATUS Andermatt Biocontrol GmbH / Probis GmbH, 96272/01-AASs ArGe GAB Biotech/IFU, Niefern-Öschelbronn, Germany GLP: yes Published: no 3687397	no	no	not protected	PKA	Y KIIIM 10.2
KMP 10.2	Dengler, D.	2002	ASSESSMENT OF TOXIC EFFECTS OF GRANUPOM ON AQUATIC PLANTS USING THE DUCKWEED LEMNA GIBBA Andermatt Biocontrol GmbH / Probis GmbH, 20011323/01-AALg ArGe GAB Biotech/IFU, Niefern-Öschelbronn, Germany GLP: yes Published: no 3687399	no	no	not protected	PKA	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 1300695 / 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 1914013	no	no	not protected	PKA	Y KIIIM 10.3
KMP 10.4	Kühner, C.	2001	GRANULOSEVIRUS CPGV SC: ACUTE TOXICITY TO THE APHID PARASITOID, APHIDIUS RHOPALOSIPHI (HYMENOPTERA, BRACONIDAE) IN THE LABORATORY Andermatt Biocontrol GmbH / Probis GmbH, 96272/01-NLAp ArGe GAB Biotech/IFU, Niefern-Öschelbronn, Germany GLP: yes Published: no 1914014	no	no	not protected	PKA	Y KIIIM 10.4
KMP 10.4	Kühner, C.	1998	GRANULOSEVIRUS CPGV SC: ACUTE TOXICITY TO THE PREDATORY MITE, TYPHLODROMUS PYRI SCHEUTEN (ACARI, PHYTOSEIIDAE) IN THE LABORATORY Andermatt Biocontrol GmbH / Probis GmbH, 96272/01-NLTp ArGe GAB Biotech/IFU, Niefern-Öschelbronn, Germany GLP: yes Published: no	no	no	not protected	PKA	Y KIIIM 10.4

			1914015					
KMP 10.4	Kühner, C.	1997	GRANULOSEVIRUS CPGV SC: ACUTE TOXICITY TO THE GROUND BEETLE, POECILUS CUPREUS L. (COLEOPTERA, CARABIDAE) IN THE LABORATORY Andermatt Biocontrol GmbH / Probis GmbH, 96272/01-NLPc ArGe GAB Biotech/IFU, Niefern-Öschelbronn, Germany GLP: yes Published: no 1914016	no	no	not protected	PKA	Y KIIIM 10.4
KMP 10.5	Wachter, S.	1998a	ACUTE TOXICITY OF CPGV SC ON EARTHWORMS, EISENIA FOETIDA USING AN ARTIFICIAL SOIL TEST Andermatt Biocontrol GmbH / Probis GmbH, 96272/01-NLEf ArGe GAB Biotech/IFU, Niefern-Öschelbronn, Germany GLP: yes Published: no 3687407	no	no	not protected	PKA	Y KIIIM 10.5
KMP 10.6	Wachter, S.	1998b	ASSESSMENT OF THE SIDE EFFECTS OF CPGV SC OF THE ACTIVITY OF THE SOIL MICROFLORA Andermatt Biocontrol GmbH / Probis GmbH, 96272/01-ABMF ArGe GAB Biotech/IFU, Niefern-Öschelbronn, Germany GLP: yes Published: no 3687408	no	no	not protected	PKA	Y KIIIM 10.6