Renewal
Assessment Report

Cydia pomonella GV

Madex Twin

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

No new data were submitted for the renewal of the approval for MADEX TWIN (*Cydia pomonella* Granulovirus (CpGV)).

Madex Twin is used as a foliar spray for the control of Oriental fruit moth (*Grapholita molesta*) in stone fruits. A summary of the critical Good Agricultural Practice of Madex Twin is presented in Table B.9.1-1.

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

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 min / max
Stone fruit	F	Oriental fruit moth (*Grapholita molesta*)	Foliar spray (tractor mounted sprayer)	Before first larvae hatch from eggs*	12 / 6 days*	a) 0.3×10^{13} GV/ha b) 3.6×10^{13} GV/ha	a) 0.1 b) 1.2	800
Stone fruit	HG **		Foliar spray (Knapsack sprayer)					

* 6 - 8 sunny days, counting 2 partially sunny days as 1 day
** HG: Home garden use

B.9.1 Effects on birds

In general, it is referred to the information submitted for MADEX in RAR Vol.3 MP Madex, chapter B.9.1. Due to the high conformity of CpGV isolates and their specific mode of action, and due to the absence of side effects to be expected from the co-formulants contained in MADEX TWIN, studies performed with Granupom or MADEX are regarded to be applicable for the evaluation of effects of the MADEX TWIN on non-target organisms. For details on the different formulations please refer to Vol.4, Part C.

B.9.1.1 Toxicity, invectiveness and pathogenicity in birds

Plant protection product

No data submitted.

B.9.1.2 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, 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 TWIN 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 TWIN 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 (EFSA1). 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 TWIN 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.

[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

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[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.9.2-2 (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:

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

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

[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

Table B.9.1-2: Risk assessment for birds and mammals for exposure via drinking water (puddles) following GAP directed application of MADEX TWIN 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	3.75×10^9	7.5×10^8	2.02×10^8	$> 1.0 \times 10^{11}$	> 494
Small herbivorous mammal - vole	0.025	0.003579			1.07×10^8	$> 4.9 \times 10^{10}$	> 456

[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 TWIN.

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

In general, it is referred to the information submitted for MADEX in RAR Vol.3 MP Madex, chapter B.9.2. Due to the high conformity of CpGV isolates and their specific mode of action, and due to the absence of side effects to be expected from the co-formulants contained in MADEX TWIN, studies performed with Granupom or MADEX are regarded to be applicable for the evaluation of effects of the MADEX TWIN on non-target organisms. For details on the different formulations please refer to Vol.4, Part C.

B.9.2.1 Risk assessment for aquatic 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.
- 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, 2016, BVL no 3306490; data point KMA 8/01).

[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

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

Effects on aquatic organisms

No experimental data for MADEX TWIN 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 TWIN. Therefore, studies conducted with GRANUPOM (or Granulosevirus CpGV SC) are fully applicable to assess possible effects of MADEX TWIN on aquatic organisms. All relevant data were assessed in the EU review. Risk assessments for MADEX TWIN 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 (MADEX), 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 TWIN 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.9.2-1: 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 $EC_{50} > 1.61 \times 10^9$ 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 12 applications in one representative crop scenario for sideward (stone fruits).

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/m²

The resulting values are presented in the following table.

Table B.9.2-2: Calculation of the predicted environmental density of MADEX TWIN and CpGV in lentic water bodies (PEDsw) after 12 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 TWIN	1.393 kg product/ha	8.66	12.065 mg/m²	300 L/m²	40.2 µg/L
Cydia pomonella Granulovirus (CpGV)	3.60×10^{13} GV/ha	8.66	3.12×10^8 GV/m²	300 L/m²	1.04×10^6 GV/L

a) Accumulated application rate, assuming no degradation between applications; calculated with a density of MADEX TWIN of 1161 g/L

b) Drift value for more than 7 applications in fruit crops (late)

The maximum PED_{SW} of 1.04×10^6 GV/L (corresponding to 40.2 µg product/L) is used for the risk assessments resulting from the application in orchards (stone fruits) with 12 × 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 12 applications at 0.1 L product/ha (corresponding to 3.6×10^{13} GV/ha) in stone fruits (orchards), assuming no degradation between the applications. This results in a PED_{sw} of 1.04×10^6 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:

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

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

Table B.9.2-3: Margin of safety for aquatic organisms exposed to CpGV

Use pattern	Test organism	PED_{SW} [a)]	Endpoint	MOS
3.6×10^{13} GV/ha in orchards	*Oncorhynchus mykiss*	1.04×10^{6} GV/L	$> 2.0 \times 10^{9}$ CFU/L	1925
	Daphnia magna		$> 2.0 \times 10^{9}$ CFU/L	1925
	Scenedesmus subspicatus		$> 2.0 \times 10^{9}$ CFU/L	1925

[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 TWIN 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 TWIN is the isolate CpGV-V22. This isolate was selected from infested *Cydia pomonella* larvae using classical selection methods. In contrast to the isolate contained in MADEX (CpGV-M), CpGV-V22 is infective to larvae of both, the codling moth, *Cydia pomonella* and the oriental fruit moth, *Grapholita molesta*. Like other CpGV isolates it is not infective to other tortricid species. The isolate does not have any other characteristics differing from the typical description of the species and the representative isolate CpGV-M.

The formulations MADEX and MADEX TWIN are two representative formulations for the renewal of approval of the active substance *Cydia pomonella* Granulovirus (CpGV). MADEX Twin was not evaluated as representative formulation for the first approval of *Cydia pomonella* Granulovirus (CpGV).

In general, it is referred to the information submitted for MADEX. Due to the high conformity of CpGV isolates and their specific mode of action, and due to the absence of side effects to be expected from the co-formulants contained in MADEX TWIN, studies performed with Granupom or MADEX are regarded to be applicable for the evaluation of effects of the MADEX TWIN on non-target organisms.

B.9.3.1 Toxicity to Bees

No new studies with the representative formulation MADEX TWIN 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.

Conclusion 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 TWIN 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 TWIN 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 TWIN 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 B.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 MP B 9.3.1/1
		LD_{50} contact 48 h	> 100µg product/bee** (> 1.63 x 10^6 CpGV/bee)		
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) Laboratory acute toxicity OECD 213/214, EPPO 170 Non-GLP	LD_{50} contact 72 h	> 100 µg product/bee** (> 1.63 x 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 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 TWIN includes application in orchards (stone fruits) (0.1L product/ha). MADEX TWIN 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 TWIN 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 TWIN 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 TWIN is negligible.

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

B.9.4 Effects on arthropods other than bees

In general, it is referred to the information submitted for MADEX in RAR Vol.3 MP Madex, chapter B.9.4. Due to the high conformity of CpGV isolates and their specific mode of action, and due to the absence of side effects to be expected from the co-formulants contained in MADEX TWIN, studies performed with Granupom or MADEX are regarded to be applicable for the evaluation of effects of the MADEX TWIN on non-target organisms. For details on the different formulations please refer to Vol.4, Part C.

B.9.4.1 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, 2016; 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 TWIN 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 TWIN. Therefore, studies conducted with GRANUPOM (or Granulosevirus CpGV SC) are fully applicable to assess possible effects of MADEX TWIN on non-target arthropods other than bees. All relevant data were assessed in the EU review. Risk assessments for MADEX TWIN 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 demonstrate that CpGV as any other baculovirus and the formulated product MADEX

[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

TWIN 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.9.4-1: 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 (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[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.

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

Table B.9.4-2: Exposure Assessment for the single application rate of MADEX TWIN

Crop	**EC_{50} [GV/ha]**	**Single application rate [GV/ha]**	**MOS**
Stone fruits	$> 7.92 \times 10^{12}$	3.00×10^{12}	2.64

MOS = Margin of safety

Table B.9.4-3: MOS calculation for the accumulated application rate of MADEX TWIN

Crop	**EC_{50} [GV/ha]**	**Maximum application rate [GV/ha]**	**MOS**
Stone fruits	$> 7.92 \times 10^{12}$	3.60×10^{13}	0.220

MOS = Margin of safety

A low margin of safety is derived for the exposure to non-target arthropods after the use of MADEX TWIN after multiple applications according to GAP based on up to 12 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.

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

[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

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.

B.9.5 Effects on earthworms

In general, it is referred to the information submitted for MADEX in RAR Vol.3 MP Madex, chapter B.9.5. Due to the high conformity of CpGV isolates and their specific mode of action, and due to the absence of side effects to be expected from the co-formulants contained in MADEX TWIN, studies performed with Granupom or MADEX are regarded to be applicable for the evaluation of effects of the MADEX TWIN on non-target organisms. For details on the different formulations please refer to Vol.4, Part C.

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.

[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

Table B.9.5-1: 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[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 1.2 L product/ha in stone fruits upon foliar application, assuming 12 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 TWIN: 0.1 L product/ha (equivalent to 3.0×10^{13} GV/ha)
- Accumulated application rate (up to 12 treatments): 1.2 L product/ha, equivalent to 3.6×10^{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.

[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

Table B.9.5-2: Calculation of the predicted environmental density of MADEX TWIN and CpGV in soil (PED_{soil}) after 12 applications at 0.1 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)]***
1.393	139.32	5.00	1.5	1.858
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)]**
3.6×10^{13}	3.6×10^{9}	5.00	1.5	4.80×10^{7}

* calculated with a density of MADEX TWIN of 1161 g/L

According to the PED_{soil} calculation the expected initial density is 1.858 mg product/kg dry soil, corresponding to 4.80×10^7 GV/kg dry soil.

Risk Assessment

The acute toxicity of GRANUPOM (or Granulosevirus CpGV SC) against *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 12 applications at 0.1 L product/ha in stone fruits. The predicted environmental density in soil (PED_{soil}) was calculated as 4.80×10^7 GV/kg soil dw (corresponding to 1.858 mg product/kg soil dw) for multiple application in stone fruits, 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.9.5-3: Risk assessment for earthworms based on the accumulated application rate

Use pattern	**Test organism**	**LC_{50} [mg product/kg soil (dw)]**	**PED_{soil} [mg product/kg soil (dw)]**	**MOS**
12 × 0.1 L product/ha in stone fruits	*Eisenia fetida*	1.67×10^{10}	4.80×10^{7}	347.9

MOS = Margin of safety

The calculated MOS value is high, indicating an acceptable acute risk to earthworms after application of MADEX TWIN 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.9.6 Effects on non-target soil micro-organisms

In general, it is referred to the information submitted for MADEX in RAR Vol.3 MP Madex, chapter B.9.6. Due to the high conformity of CpGV isolates and their specific mode of action, and due to the absence of side effects to be expected from the co-formulants contained in MADEX TWIN, studies performed with Granupom or MADEX are regarded to be applicable for the evaluation of effects of the MADEX TWIN on non-target organisms. For details on the different formulations please refer to Vol.4, Part C.

B.9.6.1 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, 2016, 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 TWIN 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 TWIN. Therefore, studies conducted with GRANUPOM (or Granulosevirus CpGV SC) are fully applicable to assess possible effects of MADEX TWIN on soil micro-organisms. All relevant data were assessed in the EU review. Risk assessments for MADEX TWIN 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.9.6-1: 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	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

[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

SC; 2.2×10^{13} GV/L*)*	N		& EFSA Journal 2012;10(4):2655[12]
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]
	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 12 applications at 0.1 L product/ha in stone fruits. The predicted environmental density in soil (PED_{soil}) was calculated as 4.80×10^{7} GV/kg soil dw (corresponding to 1.858 mg product/kg soil dw) for multiple application in stone fruits, assuming a worst case scenario that no interception and no degradation occurs between applications.

Table B.9.6-2: Risk assessment for soil micro-organisms

Use pattern	Test organism	PED_{soil} [GV/kg soil (dw)]	Endpoint [GV/kg soil (dw)]
12 × 0.1 L product/ha in stone fruits	Soil microorganism	4.80×10^{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 TWIN 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.9.7 Additional studies

No additional studies are provided.

B.9.8 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 10.3	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,	no	no	not protected	SIP	Y KIII M 10.3

			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					
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	open	no	not protected	PKA