Renewal Assessment Report

Cydia pomonella GV

Carpovirusine

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

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Rapporteur Member State: Germany

Co-Rapporteur Member State: The Netherlands

Version history

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

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B Summary, evaluation and assessment of the data and information

B.9 Effects on non-target organisms

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

No new data were submitted for the renewal of the approval for CARPOVIRUSINE (*Cydia pomonella* Granulovirus (CpGV-M)).
Carpovirusine is used as a foliar spray for the control of Codling moth (*Cydia pomonella*) Oriental fruit moth (*Grapholita molesta*) in pome fruits, stone fruits and walnut. A summary of the critical Good Agricultural Practice of Carpovirusine is presented in Table 9-1:

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

Crop and/ or situation	Table B. 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 min / max
Pome fruit Stone fruit Walnut	F	Codling moth (*Cydia pomo-nella*)	Foliar spray (tractor drawn)	BBCH 71-89	a) 3-10 (10) b) 3-10 (10)	a) 1 * b) 10	a) 1×10^{13} GV/ha b) 10×10^{13} GV/ha	1000
Pome fruit Stone fruit Walnut	HG **	Oriental fruit moth (*Grapholita molesta*)	Foliar spray (Knapsack sprayer)					

*This application rate of 1 L/ha corresponds to 0.1 L/hL in 1000 L water/ha or 0.7 L/ha LWA (leaf wall area)
** 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 preparation CARPOVIRUSINE 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.
One short-term dietary parthogenicity toxicity study with the product CARPOVIRUSINE was submitted by Arysta Lifescience S.A.S. (see below [redacted] 1993, BVL no 3689620, MP 10.1). The study confirms the reports from literature excluding adverse effects on birds (see Anonymous, 2016, BVL no 3306490; data point KMA 8/01).

B.9.1.1 Toxicity, invectiveness and pathogenicity in birds

Plant protection product

Reference:	[redacted](1993): Carpovirusine: An avian oral pathogenicity and toxicity study in the northern bobwhite; unpublished report no. 347-105, BVL no 3689620
Guideline:	FIFRA Guideline 154A-16
GLP:	Yes [redacted]
Material and methods:	
Test substance:	CARPOVIRUSINE; purity: 10^{13} CpGV/L
Test species:	Northern bobwhite (*Colinus virginianus*); 19 days of age at test initiation
Number of test animals:	Six replicates of five birds
Treatments:	1) Negative Control birds were administered deionized water at 10 ml/kg of body weight per day for five days; 2) Heat attenuated Carpovirusine was administered at approximately 10,000 mg/kg of body weight per day for five days (approximately 50,000 mg/kg total); 3) Carpovirusine was administered at approximately 10,000 mg/kg of body weight per day for five days (approximately 50,000 mg/kg total)
Duration:	5 d exposure period, 30 days observation period
Test conditions:	Food: Game bird ration formulated by Wildlife International Ltd ad libitum, Vitamin supplemented water ad libitum; Temperature: 22.3°C ± 0.6°C; Photoperiod: 16 hours of light per day; average of 589 lux of illumination; Humidity: 23% ± 9%
Deviations from guideline:	None
Endpoint:	mortality, body weight, feed consumption, clinical signs of toxicity, abnormal behaviour
Observations:	Birds were observed daily during the 30-day testing period

Results:

Carpovirusine was administered to young northern bobwhite by oral gavage at approximately 10,000 mg/kg/day for a five-day period (approximately 50,000 mg/kg total). There were no clinical signs of toxicity or apparent effects upon survival of the young northern bobwhite. No evidence of pathogenicity or replication of the test substance was observed during gross necropsy at the termination of the test. The no observed effect dosage of Carpovirusine administered to northern bobwhite in this study was 10,000 mg/kg/day for five days (approximately 50,000 mg/kg total).

A summary of endpoints is given in the table below.

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

Test species	Northern bobwhite (*Colinus virginianus*)
Toxicity of plant protection product	No signs of toxicity/infectivity/pathogenicity
	NOEL = 10000 mg/kg/day (equivalent to 1×10^{11} granules/kg bw/day), for a five-day period (approximately 50000 mg/kg total).

Comments by the RMS (2019):

The study is acceptable.

The no-observed-effect-level (NOEL) in this short-term dietary study was 10000 mg/kg/day (equivalent to 1×10^{11} granules/kg bw/day), for a five-day period.

B.9.1.2 Risk assessment for birds

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) and the max. total rate per crop/season is identical.
- 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

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

The short-term toxicity of CARPOVIRUSINE to *Colinus virginianus* was evaluated (please refer to the OECD Dossier, Doc IIIM, Section 6, Point IIIM 10.1 and EFSA Journal 2012;10(4):2655[1]). The test substance was administered at a daily dose of 10000 mg/kg bw/day for five days. No treatment related mortalities or effects occurred in the test organism. The acute LD_{50} can be determined to lie above the tested concentration of 10000 mg/kg bw (equivalent to 1.0×10^{11} GV/kg bw).

The short-term toxicity study of CARPOVIRUSINE to rats was evaluated (please refer to the OECD Dossier, Doc. IIM, Section 3, Point IIM 5.3.2 and EFSA Journal 2012;10(4):2655[1]). The study investigated the effects of an oral gavage of CpGV to Sprague-Dawley rats. No test substance related signs of infectivity were observed in the study, so that the acute oral LD_{50} was estimated to be > 5000 mg/kg bw (equivalent to 4.9×10^{10} GV/kg bw).

All available data for birds and mammals indicate that CARPOVIRUSINE is not toxic, not pathogenic or infective to birds or mammals. Nevertheless, a quantitative risk assessment confirming the safe use is provided.
The EU agreed endpoints are summarised in the table below.

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	OECD Dossier, Doc M, IIIM,

[1] 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

		(equivalent to 1.0×10^{11} GV/kg bw) LD_{50} > 10 000 mg CARPOVIRUSINE (10^{11} granules/kg bw) No indications for infectivity or pathogenicity based on visual gross necropsy	Section 6, Point IIIM 10.1 & EFSA Journal 2012;10(4):2655[1]
CARPOVIRUSINE	Rat, acute oral	LD_{50} > 5000 mg/kg bw ($LD_{50} > 4.9 \times 10^{10}$ GV/kg bw)	OECD Dossier, Doc. IIM, Section 3, Point IIM 5.3.2 & EFSA Journal 2012;10(4):2655[1]

The available endpoints for birds and mammals indicate no toxicity or pathogenicity of *Cydia pomonella* Granulovirus (CpGV) independently of the study design. 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 of the 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)[2] 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[3] 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[3]. 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.9.2-3 (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:

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

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:

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

Based on the available data, the MOS values of birds and mammals for CpGV were calculated in the following table.

Table B.9.1-2: Risk assessment for birds and mammals for exposure via drinking water (puddles) following GAP directed application of CARPOVIRUSINE in orchards in accordance with SANCO 4145/2000[4]

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	1.0×10^{10}	2.0×10^{9}	5.39×10^{8}	$> 1.0 \times 10^{11}$	> 185
Small herbivorous mammal - vole	0.025	0.003579			2.86×10^{8}	$> 4.9 \times 10^{10}$	> 171

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

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

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.

[4] European Commission, 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

B.9.2 Effects on aquatic organisms

The following information 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] (1994a): Test to evaluate acute toxicity (96 Hours) in Freshwater fish (*Brachydanio rerio*) using a static method; unpublished report no. E150, BVL no 3689633
Guideline:	OECD Guideline No. 203 (1984) and EEC Directive 92/69 – Method C.1 (1992)
GLP:	Yes
Material and methods:	
Test substance:	CARPOVIRUSINE; purity: 10^{13} CpGV/L
Test species:	Zebra fish (*Brachydanio rerio = Danio rerio*); length: 3.0 - 3.6 cm (mean 3.3)
Number of test animals:	Control group: 10, Treated group: 50
Treatments:	0, 1, 10, 50, 100 and 250 mg/L
Duration:	96 hours
Test conditions:	Static system; Temperature from 19.6 to 20.7°C; photoperiod: 12-hour; Oxygen content: At least 76% of the air saturation value; pH: 6.75 - 7.63
Deviations from guideline:	- One from ten measured fishes was larger than 3.5 cm. - Slight deviation of temperature was noted beyond the norms, with a minimum at 18.9°C. - Temperature variation was greater than ± 1°C in the control medium. These deviations were not considered to have affected the outcome or the objectives of the study.
Endpoint:	Mortality, sublethal effects
Observations:	Daily check for mortality, occurrence of sublethal effects (loss of equilibrium, erratic swimming loss of reflex, excitability, discolouration, or change in behaviour), dissolved oxygen, pH and temperature

Results:

No mortality was occurred during the 96-hour period, up to the concentration of 250 mg/L. Therefore, it was not possible to determine LC_{50} value at 96 hours which can be considered higher than 250 mg/L. At the concentration of 250 mg/L, a pale pink coloration was observed during all the study. No other effect was observed whatever the concentration or the observation stage.

A summary of endpoints is given in the table below.

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

Test species	*Danio rerio*
Toxicity of plant protection product	No signs of toxicity/infectivity/pathogenicity
	$LC_{50} > 250$ mg/L

Comments by the RMS (2019):

The study is acceptable.
The acute 96-hour LC_{50} of Zebra fish in a static system is higher than 250 mg/L. A NOEC of 100 mg/L

can be determined.

B.9.2.2 Effects on freshwater invertebrates

Plant protection product

Reference:	[redacted] (1994b): Test to evaluate acute toxicity (48 hours) in *Daphnia*; unpublished report no. E151, BVL no 3689641
Guideline:	OECD Guideline No. 202 (1984) and EEC Directive 92/69 – Method C.2 (1992)
GLP:	Yes
Material and methods:	
Test substance:	CARPOVIRUSINE; purity: 10^{13} CpGV/L
Test species:	Control group: 20, Treated group: 101
Number of test animals:	*Daphnia magna*
Treatments:	0, 1, 10, 50, 100 and 250 mg/L
Duration:	48 hours
Test conditions:	Static system; Temperature: 23 - 21.2°C; Photoperiod: Darkness; Oxygen content: At least 7.8 mg per litre; Hardness: 180 mg CaCO3/L; pH: 7.61 - 7.76
Deviations from guideline:	- At 50 mg/L a volume of test medium of 1.7 mL instead of 2 mL. - 6 Daphnia in one tube instead of 5. - At the start of the range-finding study, the age of daphnia was 24 hours and 15 minutes. 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:

No mortality was occurred during the 48-hour period, up to the concentration of 250 mg/L. Therefore it was not possible to determine EC_{50} value at 48 hours which can be considered higher than 250 mg/L. 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

Comments by the RMS (2019):

The study is acceptable.
The acute 48-hour EC_{50} of *Daphnia magna* in a static system is higher than 250 mg/L.

B.9.2.3 Effects on algae growth

Plant protection product

Reference:	Pawlowski, S., Wydra, V., Vinken, R. (2007): Toxicity of Carpovirusine to *Pseudokirchneriella subcapitata* in an Algal Growth Inhibition Test Final Report; unpublished report no. 26191210, BVL no 3689706
Guideline:	OECD Guideline No. 201

GLP:	Yes
Material and methods:	
Test substance:	CARPOVIRUSINE; purity: 10^{13} CpGV/L
Test species:	*Pseudokirchneriella subcapitata*; Chodat, Strain No. 61.81 SAG
Number of test animals:	n.a. (initial cell density: 5000 algal cells per mL)
Treatments:	0, 0.95, 3.05, 9.77, 31.25 and 100 mg/L
Duration:	72 hours of exposure
Test conditions:	static system; Temperature: 23°C ± 2°C; Photoperiod: Continuous illumination with a light intensity of 4440 to 8880 Lux with a maximum deviation of ± 15%; Test units: Erlenmeyer flasks of 50 mL volume with 50 mL test medium; pH: 8.1 - 8.2 at test start and pH 9.6 - 9.7 at test end
Deviations from guideline:	none
Endpoint:	biomass and growth rate
Observations:	daily cell density measurements and daily check for test conditions; microscopic examination of the shape of the algal cells

Results:

The experiment is valid because:
The cell density in the control cultures increased by a factor of 158 within 72 hours. Thus, the validity criterion of an increase factor of at least 16 within 72 hours is fulfilled.
The coefficient of variation on the sectional (daily) growth rates in the control cultures during the course of the test was 27.7%. Thus, the validity criterion of maximum 35% is fulfilled.
The coefficient of variation of average growth in replicate control cultures was 1.9%. Thus, the validity criterion of maximum 7% is fulfilled.
The 72-hour EC_{50} values were above 100 mg test item/L for biomass and growth rate, respectively. The EC_{10} values were calculated to be above 100 mg test item/L for biomass and growth rate, respectively.
The 72-hour NOEC was determined to be at least 100.0 mg test item/L. The 72-hour LOEC was determined to be above 100.0 mg test item/L.
At the microscopic examination of the shape of the algal cells after 72 hours test period no difference was observed between the algae growing in the test concentration of nominal 100 mg test item/L and the algal cells in the control. Thus, the shape of the algal cells growing at least up to this test concentration was obviously not affected.
In the control the cell density has increased from nominal N = 5.0×10^3 cells/mL at the start of the test (0 hours) to N = 7.9×10^5 cells/mL (mean value) after 72 hours by a factor of approximately 158. Thus, the algal growth in the control was sufficiently high under the test conditions.

A summary of endpoints is given in the table below.

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

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

Comments by the RMS (2019):

The study is acceptable.
The 72-hour EC_{50} values were above 100 mg test item/L for biomass and growth rate, respectively.
The 72-hour NOEC was determined to be at least 100 mg test item/L.

Reference:	Jehle, J., Matt-Schmid A. (2006): Analytical phase report: Toxicity of Carpovirusine to Pseudokirchneriella subcapitata in an Algal Grotwh Inhibition Test; unpublished report no. 26191210, BVL no 3689555
Guideline:	SANCO/825/00 rev. 7 (17/3/2004): European Commission, Directorate General Health and Consumer Protection (2004). Guidance document on residue analytical methods
GLP:	Yes
Material and methods:	
Test substance:	1) CARPOVIRUSINE; purity: 10^{13} CpGV/L; Batch no. 1548/Cfr; 2) Reference item: Carpovirusine Technical Concentrate; Batch: 1560/Technical mother suspension; (The Reference Item CpGV (Neustadt) was used as a reference (posi-tive control) during Real Time PCR and as a standard for the melting curve analysis. The Reference Item CpGV (Neustadt) was taken from the Test Site)
Test species:	n.a.
Number of test animals:	n.a.
Treatments:	See text below
Duration:	See text below
Test conditions:	See text below
Deviations from guideline:	n.a.
Endpoint:	n.a.
Observations:	n.a.

MATERIAL AND METHODS

Test Item

Designation	CARPOVIRUSINE
Purity	10^{13} CpGV/L
Characteristics	Red liquid, suspension concentrate
Batch no.	1548/Cfr
Expiration date	Not applicable

Reference Item

Product	Carpovirusine Technical Concentrate
Batch	1560/Technical mother suspension
Storage	At -18°C until 29/12/2007
Expiry date	At 4°C until 29/06/2006
Number of Granulosvi-rus/L (Petroff-Hausser-Chamber)	7.4 × 1013 ± 8.7
Number of Granulosvi-rus/L (Biological titration)	3.8×10^{13} [min 3.1×10^{13} ; max 4.7×10^{13}]
Appearance	Olive brown
Certificate of analysis	Dated January 23 2006 Natural Plant Protection (N.P.P.) 35 avenue Léon Blum Parc d'activités Pau Pyrénées

	64000 PAU France
Use	The Reference item Carpovirusine Technical Concentrate was used for generating the standard curve of the Real Time PCR. It is an unformulated CpGV suspension in water produced by extraction from dead bodies of infected larvae collected with the artificial diet in which they are reared.
Product	Technical Product of *Cydia pomonella* Granulovirus (CpGV)
Batch	TPCpGVBTPS_01
Storage	-20°C or colder
Expiry date	March 2007
Number of Granulosvirus/L	1.5×10^{14}
Appearance	White-light cream, liquid
Certificate of analysis	Dated March 10, 2005 Phytomedizin / Biotechnologischer Pflanzenschutz Dienstleistungszentrum Ländlicher Raum (DLR) Rheinpfalz Breitenweg 71 67435 Neustadt / Weinstraße Germany
Use	The Reference Item CpGV (Neustadt) was used as a reference (positive control) during Real Time PCR and as a standard for the melting curve analysis. The Reference Item CpGV (Neustadt) was taken from the Test Site.

Specimens

For the verification of the test Item Concentrations in the test solutions the Test Site received following specimens on 11.01.2006 from the Test Facility. All specimens were stored at -18°C or colder at the Test Site.

Study Design and Methods

Generating the standard curve	Prior to generating the standard curve, the average weight of one *Cydia pomonella* Granulovirus Occlusion Body (CpGV OB) had to be determined. This determination was done as a part of the Study ARY04 "Validation of an analytical method for determination of *Cydia pomonella* Granulovirus (CpGV) used in Carpovirusine (Technical Concentrate) in surface water" and is described in detail in the Final Report of ARY04. In short, 1.5 mL of the Reference Item CpGV (Carpovirusine, Technical Concentrate) was purified using a SDS/ultrasonic water bath- and sandwich filter treatment followed by a glycerol layer gradient. The purified CpGV OB pellet was resuspended in 1.5 mL sterile water and the CpGV OB concentration was enumerated in the Petroff-Hausser counting chamber. The CpGV OBs in 1 mL of this suspension were dried in a speed-vac and its weight was determined with an analytical balance. Out of 6 independent measurements the average weight of one CpGV OB was calculated with 9.24E-15 g (SD 2.62E-15). Based on this calculation, the standard curve was generated. Different water samples containing known amounts of CpGV OB were created in the range of CpGV OB concentrations in the algal water specimens expected. The calculated value of the nominal concentration (CpGV OB) that is expected in the algal water (100 mg/L) was 9.8E+8 CpGV OB/L.

	1 mL of each specimen was used for the preparation and viral DNA isolation. Due to the Qiagen Kit the whole viral DNA of each specimen was solved in 400 µL AE buffer of which 5 µL was used for one Real Time PCR reaction (9.8E+05 CpGV/OB/mL × 5 µL/400 µL). Thus, 12250 CpGV Obs per one Real Time PCR reaction equal a nominal test item concentration of 100 mg/L. The established standard curve to determine the amount of CpGV Obs in the algal water specimens was 2.59E+07, 8.64E+07, 2.59E+08, 7.78E+08, 8.64E+08, 1.73E+09, 2.59E+09 and 8.64E+09 CpGV OB/L Reference Item (CpGV Carpovirusine, technical concentrate). The Reference Item CpGV (Neustadt) was used as 8.64E+09 CpGV OB/L as a positive control.
Preparation of the specimens	For using the specimens in the Real Time PCR reactions, the CpGV Obs in the specimens had to be purified and concentrated. Three independent repeats were done for all specimens. The same protocol was used for preparing the standard solutions as well as for the Reference Item CpGV (Neustadt).
Viral DNA isolation	Prior using Real Time PCR, the CpGV DNA of the standard solutions, of the Reference Item CpGV (Neustadt) and of the specimens had to be isolated. To solubilise the CpGV Obs 10 µL Na_2CO_3 (1 M) was added to 100 µL of the prepared samples and the CpGV solution was readjusted with 15 µL HCL (1 M) to pH 8. The pH-value was checked with pH-paper before adding 3 µg herring sperm DNA. The viral DNA isolation was done with a Qiagen Kit.
Real Time PCR	The amount of CpGV Obs in the specimens was detected with prior isolation of the viral DNA. Using oligonucleotides specific of the CpGV granulin gene, a 422 bp DNA fragment was amplified specifically. For accurate quantification of the amplified DNA the fluorescent dye SYBR Green was used. In the reaction set up used in this study, 5 µL of the standard solutions (CpGV Carpovirusine, Technical Concentrate), the Reference Item CpGV (Neustadt) as well as the specimens were added to 35 µL of the PCR reaction mix. The PCR conditions and the used programme in the quantitative reaction are below mentioned. The Real Time reactions were performed in 8 Strips Low Profile Tubes.
Specificity of the Real Time PCR method	The quantitative detection of CpGV was performed with a Real Time PCR. For the amplification of a CpGV specific DNA fragment, granulin gene specific oligonucleotides were used. The obtained amplified PCR product is a 422 bp DNA fragment. The specificity of the Real Time PCR method and thus of the amplified PCR product was tested using melting curve analysis. The melting temperature ™, based on the length and the GC content of the specific 422 bp CpGV granulin fragment, is 83°C as shown with the purified CpGV standard from the Reference Item CpGV (Neustadt). As expected, in the controls without viral DNA in the PCR no CpGV specific peak was detected in the melting curve.
Linearity of the Real Time PCR method	A standard curve (plot of C(T) cycle against different CpGV concentrations (log LOQ)) was generated using following standard dilutions: 2.59E+07, 8.64E+07, 2.59E+08, 7.78E+08, 8.64E+08, 1.73E+09, 2.59E+09 and 8.64E+09 CpGV OB/L Reference Item (CpGV Carpovirusine, technical concentrate). For each concentration Real Time PCR reaction was performed twice. The standard dilution 1.73E+09 CpGV OB/L did not follow the linear regression of the other n times CpGV OB/L and was disregarded in the linearity analysis. The other reactions resulted in a standard curve (y = -0.25 x + 9.89) with a correlation coefficient of R2 = 0.980. This high correlation coefficient shows a strong linearity between the amount of different CpGV concentrations in the reaction and the determined C(T) cycle.

Results:

Three independent repeats of the algal water (100 mg/L) taken at time t = 0 h and t = 72 h were treated as described in the protocol before and were quantified in the Real Time PCR. For the quantification a standard curve was generated (2.59×10^7, 8.64×10^7, 2.59×10^8, 7.78×10^8, 8.64×10^8, 1.73×10^9, 2.59×10^9 and 8.64×10^9 CpGV OB/L Reference Item Carpovirusine Technical Concentrate) and Real Time PCR was performed twice. In the Real Time PCR a linear relationship exists between the log quantity of the sample and the PCR Cycle time C(T) at which the fluorescence in the PCR exceeds a certain threshold line. The threshold was adjusted for the obtained quantification concentration to the value at which the correlation coefficient R^2 of the standard line was at its maximum. At this threshold [maximum of R^2] the statistical error of the measurement was at its minimum. In all specimens the same fluorescence exceeded the baseline at C(T) cycle of 21.13 to 22.13.
The purified unformulated Reference Item (Carpovirusine Technical Concentrate) was used as a quantification standard. In order to quantify formulated Test Item (Carpovirusine, batch n°1548/Cfr) a standard curve of purified unformulated Reference Item (Carpovirusine Technical Concentrate) was generated and compared to the formulated Test Item. There, 1.225×10^7 CpGV Obs/L of the purified unformulated Reference Item (Carpovirusine Technical Concentrate) correlated with 4.739×10^6 CpGV Obs/L of the formulated Test Item. Since the purified unformulated Reference Item (Carpovirusine Technical Concentrate) was used Reference Item (Carpovirusine Technical Concentrate) to quantify formulated test item a correction factor of $4.739 \times 10^6 / 1.225 \times 10^7 = 0.3869$ was applied. The corrected quantified CpGV and their percentage of the nominal concentration for the algal water specimens taken on different times was in the range between 125.35% (t = 0 h, specimen 5) and 83.93% (t = 72 h, specimen 8). The mean percentage of the nominal concentration for the duplicate algal water specimens taken on time t = 0 h was 117.50% and on time t = 72 h was 88.43%.
The CpGV concentration was stable over the test period and could be detected in the algal water (100 mg/L) specimens at the expected nominal concentration of 100 mg/L corresponding to 9.8×10^8 CpGV/L. The SANCO guideline requires a mean recovery rate in the range of 70 - 110%. Thus, the percentage of the nominal concentration at time t = 0 h is slightly higher whereas at time t = 72 h the proposed 70 - 110% is fulfilled.
For the quantification of CpGV in control algal water three independent repeats of the specimens taken on each time point (t = 0 h and t = 72 h) were treated as described in the above paragraph Study Design and Methods. A standard curve (2.59×10^7, 8.64×10^7, 2.59×10^8, 7.78×10^8, 8.64×10^8, 1.73×10^9, 2.59×10^9 and 8.64×10^9 CpGV OB/L Reference Item CARPOVIRUSINE Technical Concentrate) was generated twice. Using this standard curve, the amount of CpGV in the control algal water specimens was quantified. For these specimens, the fluorescence exceeded the baseline at C(T) cycle of 27.30 to 34.40.
The specificity of these amplified PCR products were tested using melting curve analysis. The detected melting temperature of these PCR products obtained from reactions with control algal water specimens and the granulin specific oligonucleotides was 83°C. In comparison with the melting point analysis performed on the PCR product obtained from reactions with the granulin specific oligonucleotides and the Reference Item CpGV (Neustadt) as template, the same melting temperature of 83°C was detected. This result indicated that the control algal water specimens were somehow contaminated with CpGV. In the control reactions without viral DNA in the PCR no CpGV specific peak was detected in the melting curve.
It has to be noticed that application of Real Time PCR is extremely sensitive to small contaminations since the amplification rate of the initial DNA amount in the sample is $2^{C(T)}$ for a given C(T) value. C(T) values between 27 and 35 as observed in the control algal water reactions based on a 2^{27} ($= 13.4 \times 10^7$) to 2^{35} ($= 17.2 \times 10^9$) fold amplifications of initial DNA. The smallest trace amount of CpGV DNA can be detected. The mean percentage of the nominal concentration for the control algal water specimens taken on different times was in the range between 0.19% (t = 0 h) and 0.93% (t = 72 h). For the control algal water specimens taken at time t = 72 h a low C(T) value was measured in always one of the three repeats (specimen 72-3/2 and specimen 72-4/2), which indicates some contamination with CpGV in these samples. Since al1 other control algal water specimens taken at time t = 0 h and t = 72 h showed high C(T) values (= low CpGV concentrations) it is most likely that these control algal water samples 72-3/2 and 72-4/2 were contaminated with CpGV DNA during viral DNA preparation and do not reflect the real CpGV concentration at time t = 72 h. Ignoring these two measurements the mean percentage of

the nominal concentration for the control algal water specimens taken at time t = 72 h is 0.09%. Therefore, it is concluded that the control algal water specimens were free of CpGV.

Study conclusion:

In general, there are three basic features, which make this quantitative detection technique extremely specific and sensitive. The first one is the utilisation of CpGV sequence-specific oligonucleotides for the PCR amplification of the template of interest. In this study, CpGV granulin gene specific oligonucleotides were used for the amplification of a CpGV specific DNA fragment. The obtained amplified PCR product is a 422 bp DNA fragment. Second, the identity of the amplified PCR product was analysed using melting curve analysis. The melting temperature ™ is based on the length and the GC content of the PCR product. For the CpGV specific 422 bp granulin fragment, the melting temperature is 83°C as shown with the purified CpGV standard from the Reference Item CpGV Neustadt). Third, the intercalating SYBR Green dye was used for DNA quantification. SYBR Green dye fluorescence only when bound to double-stranded DNA products generated by PCR. The design of highly specific primers and optimised reagents insure sensitive quantification.
All algal water specimens (100 mg/L CARPOVIRUSINE) analysed in this study contained CpGV Obs in a concentration between 1.15E+09 CpGV/L to 8.67E+08 CpGV/L. The CpGV concentration over the test period is rather stable. According to the guideline outlined in SANCO/825/00 rev. (17/3/2004) the recovery rate of a quantitative method should be in the range of 70 - 110%. This guideline was developed for chemical pesticides and was followed in this study because no appropriate guideline exists for microbial pesticides. The mean percentage of the nominal concentration at time t = 0 h (117.50%) is slightly higher whereas at time t = 72 h (88.43%) it fills in the 70 - 110% range. Given that the measured C(T) values during the Real Time PCR correlates to the logarithm of concentrations applied in the PCR reaction, very small variations in the C(T) value results in a high variation in accuracy, which contributes to the variations in the recovery rate. For example, the algal water (100 mg/L CARPOVIRUSINE) taken at time t = 0 h the lowest C(T) cycle was measured at 21.13 using the algal water specimen 0-5/2. The corresponding nominal concentration of this C(T) value is 130.61%. The highest C(T) value was determined at 21.76 using the algal water specimen 0-6/1. The corresponding nominal concentration of the C(T) value at 21.76 is 90.81% whereas the difference of these two C(T) cycles is only 0.63. For the algal water (100 mg/L CARPOVIRUSINE) taken at time t = 72 h the measured C(T) values were in the range between 21.25 (specimen 72 7/3) and 22.13 (specimen 72 7/1). The corresponding nominal concentration of the C(T) value of 21.13 is 73.47% whereas at a C(T) cycle of 21.25 a nominal concentration of 121.43% was determined. The established Real Time PCR method in this analytical phase is the most precise and accurate method available.
It has to be noticed that application of Real Time -PCR-is extremely sensitive to small contaminations since the amplification rate of the initial DNA amount in the sample is $2^{C(T)}$ to a given C(T) value. In the control algal water C(T) values were measured in the range between 27and 34. This correlates from 2^{27} (= 13.4×10^7) to 2^{35} (= 17.2×10^9) fold amplifications of initial DNA. Therefore, the smallest traces of CpGV DNA can be detected. The mean percentage of the nominal concentration for the duplicate control algal water specimens taken at time t = 0h and t = 72 h was 0.19% and 0.93%, respectively. A low C(T) cycle always measured in only one of the three repeats using the control algal water specimens taken at time t = 72 h (specimen 72-3/2 and specimen 72-4/2) indicates some CpGV contamination in these samples. Since in al1 other control algal water specimens taken at time t = 0 h and t = 72 h high C(T) values were measured it could be possible that these samples were contaminated during viral DNA preparation. Thus, the mean percentage of the nominal concentration for the duplicate control algal water specimens taken at time t = 72 h does not reflect the true CpGV concentration. If these two measurements are not included into the calculation the mean percentage of the nominal concentration for the duplicate control algal water specimens taken at time t = 72 h is 0.09%. Accordingly, the control algal water can be considered without CpGV.

Comments by the RMS (2019):

The study is acceptable.
According to the guideline SANCO/825/00 rev. (17/3/2004) the recovery rate of a quantitative method

should be in the range of 70 - 110%. In all algal water specimens were detected at the expected concentration of 100 mg/L of Carpovirusine corresponding to 9.8×10^8 CpGV/L. The mean determined CpGV concentrations were in the range of 1.15×10^9 CpGV/L (t = 0 h) to 8.67×10^8 CpGV/L (t = 72 h), which corresponds to a mean percentage of the nominal concentration of 117.50% (t = 0 h) to 88.43% (t = 72 h). The CpGV concentration over the test period was rather stable.

B.9.2.4 Effects on plants other than algae

No data were submitted.

B.9.2.5 Risk assessment for aquatic organisms

Table B.9.2-1: Summary of the studies on toxicity on aquatic organisms treated with toxin/metabolite from the active ingredient or the plant protection product CARPOVIRUSINE.

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					
Zebra fish (*Brachydanio rerio* = *Danio rerio*)	96 hours	0, 1, 10, 50, 100 and 250 mg/L	LC50 > 250 mg/L, NOEC = 100 mg/L	No signs of toxicity/pathogenicity	[redacted] (1994a), BVL no 3689633
Water flea (*Daphnia magna*)	48 hours	0, 1, 10, 50, 100 and 250 mg/L	EC50 > 250 mg/L, NOEC ≥ 250 mg/L	No signs of toxicity/pathogenicity	[redacted] (1994b), BVL no 3689641
Green algae (*Pseudokirchneriella subcapitata*)	72 hours	0, 0.95, 3.05, 9.77, 31.25 and 100 mg/L	EC50 > 100 mg/L, NOEC ≥ 100 mg/L	No signs of toxicity/pathogenicity	Pawlowski, S., Wydra, V., Vinken, R. (2007), BVL no 3689706

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) and the max. total rate per crop/season is identical.
- As can be seen from the initial DAR (2008), risk quotients (Margin-of-Safety-values) clearly exceeded the default trigger values.
- Literature search submitted for the renewal of the approval for CpGV did not indicate any adverse effects on aquatic organisms associated with the use of baculoviruses (see Anonymous, 2016, BVL no 3306490; data point KMA 8/01).

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

Effects on aquatic organisms

Effects of the formulation CARPOVIRUSINE on aquatic organisms have been assessed for the first submission. Therefore, all relevant data were assessed in the EU review. Risk assessments for CARPOVIRUSINE 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 CARPOVIRUSINE to *Danio rerio, Daphnia magna and Pseudokirchneriella subcapitata* was evaluated (please refer to the OECD Dossier, Doc IIIM, Section 6, Point IIIM 10.2 and EFSA Journal 2012;10(4):2655[5]).

All available data for aquatic organisms demonstrate that CpGV as any other baculovirus and the formulated product CARPOVIRUSINE 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.9.2-2: 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** **$LC_{50} > 1.0 \times 10^9$ GV/L**	OECD Dossier, Doc M, IIIM, Section 6, Point IIIM 10.2 & 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 > 2.0×10^9 GV/L	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** **$EC_{50} > 1.0 \times 10^9$ GV/L**	OECD Dossier, Doc M, IIIM, Section 6, Point IIIM 10.2 & EFSA Journal 2012;10(4):2655[6]
GRANUPOM (as Granulosevirus CpGV SC; 2.2×10^{13} GV/L)	*Daphnia magna*	48-hour (static) EC_{50} > 100 mg/L EC50 > 2.0×10^9 GV/L	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** **$EC_{50} > 1.0 \times 10^9$ GV/L**	OECD Dossier, Doc M, IIIM, Section 6, Point IIIM 10.2 & EFSA Journal 2012;10(4):2655[6]

[5] 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

[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) EC_{50} > 100 mg/L **EC50 > 2.0×10^9 GV/L**	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, stone fruits and walnut). The predicted environmental density of CpGV in lentic water bodies (PEDsw) is calculated as

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

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

The resulting values are presented in the following table.

Table B.9.2-3: Calculation of the predicted environmental density of CARPOVIRUSINE and CpGV in lentic water bodies (PED_{sw}) after 10 applications at 1.0 L product/ha

	Application rate [a)]	**Relevant drift rate [%]** [b)]	**Amount reaching the water**	**Water volume (30 cm water layer)**	**Initial PED_{SW}**
CARPOVIRUSINE	10.472 kg product/ha	8.66	90.688 mg/m²	300 L/m²	302.3 µg/L
Cydia pomonella Granulovirus (CpGV)	1.0×10^{14} GV/ha	8.66	8.66×10^8 GV/m²	300 L/m²	2.89×10^6 GV/L

a) Accumulated application rate, assuming no degradation between applications; calculated with a density of CARPOVIRUSINE of 1.0472 g/cm³
b) Drift value for more than 7 applications in fruit crops (late)

The maximum PED_{SW} of 2.89×10^6 GV/L (corresponding to 302.3 µg product/L) is used for the risk assessments resulting from the application in orchards (pome fruits, stone fruits and walnut) with 10 × 1.0 L product/ha.

Risk Assessment

Aquatic organisms may be exposed to CpGV entering surface waters via spray drift. As stated above, the exposure calculation was based on a worst-case scenario following 10 applications at 1.0 L product/ha (corresponding to 1.0×10^{13} GV/ha) in pome fruits, stone fruits and walnut (orchards), assuming no degradation between the applications. This results in a PED_{sw} of 2.89×10^6 GV/L.

The risk of *Cydia pomonella* Granulovirus (CpGV) to aquatic organisms was assessed from margin of

safety (MOS; corresponding to TER) values according to the following equation:

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

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

Table B.9.2-4: Margin of safety for aquatic organisms exposed to CpGV

Use pattern	Test organism	PED_{SW} [a)]	Endpoint	MOS
1.0×10^{14} GV/ha in orchards	*Danio rerio*	2.89×10^{6} GV/L	> 1.0×10^{9} GV/L	346
	Daphnia magna.		> 1.0×10^{9} GV/L	346
	Pseudokirchneriella subcapitata		> 1.0×10^{9} GV/L	346

[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

CARPOVIRUSINE is a biological insecticide, formulated as suspension concentrate, containing a nominal concentration of 1×10^{13} infective granules of *Cydia pomonella* Granulovirus (CpGV) in 1 L product. The CpGV isolate contained in CARPOVIRUSINE is the Mexican isolate (CpGV-M) which acts highly specific against larvae of the codling moth, *Cydia pomonella* and the oriental fruit moth, *Grapholita molesta*.

CARPOVIRUSINE was one of the representative formulations for first approval of the active substance CpGV. Since first evaluation the formulation of CARPOVIRUSINE was slightly changed. However, these minor changes are regarded to be not hazardous to non-target organisms and the environment. Therefore, studies performed with the old formulation are suitable for the renewed risk assessment of CARPOVIRUSINE.

B.9.3.1 Toxicity to Bees

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

Report:	B 9.3.1/1 Schmitzer, S. (2006): Effects of CARPOVIRUSINE (Acute Contact and Oral) on Honey Bees (*Apis mellifera* L.) in the Laboratory, Project n° 26194035, IBA-CON, Rossdorf, Germany, BVL no 3689722
Guidelines:	OECD 213/214
GLP:	Yes
Validity:	Acceptable

Executive Summary

The product CARPOVIRUSINE was tested for its acute oral contact and toxicity on honey bees.

Five replicates, each consisting of 10 bees in one cage per test concentration were assessed of mortality after 4 - 24 and 48 hours.

The following doses were tested:

- Contact test: 100 µg product/bee
- Oral test: 108.4 µg product/bee

The reference item used is dimethoate 400 g/L (Perfekthion EC).

In the contact toxicity test, no mortality was observed at 100 µg product/bee after 48 hours. No mortality occurred in the control (water + 0.5% Adhäsit).

In the oral toxicity test, the maximum nominal test level of CARPOVIRUSINE (100 µg product/bee) corresponded to an actual intake > 108.4 µg a.s./bee. This concentration level led to no mortality after 48 hours.

No mortality occurred in the control (50% sugar solution).

No test item induced behavioural effects were observed at any time in both tests.

Therefore, contact LD_{50} (48 h) was > 100 µg product/bee and oral LD_{50} (48 h) was > 108.4 µg product/bee.

RESULTS AND DISCUSSION

Oral toxicity test:

No behavioural abnormalities attributed to exposure of the test item to the bees occurred during the experimental time of 48 hours.

There were behavioural abnormalities consistent with the observed toxicity in the toxic standard test

Contact toxicity test:

No behavioural abnormalities attributed to exposure of the test item to the bees occurred during the experimental time of 48 hours.

There were behavioural abnormalities consistent with the observed toxicity in the toxic standard test.

Table B.9.3-1: Mortality and behavioural abnormalities of the bees in the oral toxicity test

Ingested dose	After 4 hours		After 24 hours		After 48 hours	
	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.

	Mean	Mean	Mean	Mean	Mean	Mean
	%	%	%	%	%	%
Test item µg product/ bee 108.4	0.0	0.0	0.0	0.0	0.0	0.0
Water	0.0	0.0	0.0	0.0	0.0	0.0
Toxic standard µg a.s./bee						
0.22	32.0	56.0	94.0	0.0	96.0	0.0
0.16	28.0	40.0	94.0	4.0	98.0	0.0
0.11	2.0	40.0	70.0	4.0	76.0	0.0
0.08	4.0	18.0	44.0	0.0	46.0	0.0

Table B.9.3-2: Mortality and behavioural abnormalities of the bees in the contact toxicity test

Dose	After 4 hours		After 24 hours		After 48 hours	
	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.
	Mean	Mean	Mean	Mean	Mean	Mean
	%	%	%	%	%	%
Test item µg product/ bee 100.0	0.0	0.0	0.0	0.0	0.0	0.0
Water	0.0	0.0	0.0	0.0	0.0	0.0
Toxic standard µg a.s./bee						
0.30	14.0	40.0	84.0	0.0	84.0	2.0
0.20	0.0	20.0	42.0	2.0	48.0	0.0
0.15	0.0	0.0	18.0	0.0	34.0	0.0
0.10	0.0	0.0	12.0	0.0	12.0	0.0

Conclusion by the applicant

The ingestion and the contact with CARPOVIRUSINE up to 100 µg/bee have no adverse effect to honey bees in the conditions of the test.

Oral acute toxicity: LD_{50} 24 h > 108.4 µg/bee; LD_{50} 48 h > 108.4 µg/bee

Contact acute toxicity: LD_{50} 24 h > 100 µg/bee; LD_{50} 48 h > 100 µg/bee

CONCLUSION RMS:

RMS concludes the validity criteria of OECD Guideline 213 and 214 are met:

- less than 10% mortality in the control (oral toxicity test: 0% during the 48h test period; contact toxicity test: 0% mortality during the 48h test period)

- LD_{50} for the reference item was slightly below the range for the oral test (range: 0.10-0.35 µg a.s./bee; observed: 0.09 µg a.s./bee) and the contact test (range: 0.10-0.30 µg a.s./bee; observed: 0.22 µg a.s./bee); 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 CARPOVIRUSINE were submitted. However, effects of *Cydia pomonella* granulovirus on honey bees have been discussed in Volume 3 MA, B.9.3.2.

B.9.3.3 Pathogenicity to Bees

No tests regarding the pathogenicity of CARPOVIRUSINE were submitted. However, effects of *Cydia pomonella* granulovirus on honey bees have been 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 CARPOVIRUSINE 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 9.3.4. 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 CARPOVIRUSINE includes application in orchards (pome and stone fruits) and walnuts (1L product/ha). CARPOVIRUSINE contains a minimum of 1x10^{13} *Cydia pomonella* Granulovirus CpGV/L, and one application will be 0.7 L product/ha per LWA (leaf wall area).

Bees may be exposed to CARPOVIRUSINE 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 CARPOVIRUSINE 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 CARPOVIRUSINE is negligible.

Conclusion by the RMS (2019):

Based on the total set of data, it can be concluded that CARPOVIRUSINE has to be classified as non-hazardous.

B.9.4 Effects on arthropods other than bees

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

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

Plant protection product

Reference:	Hoxter K.A., Porch J.R., Krueger H.O. (1999a): Carpovirusine: A dietary pathogenicity and toxicity study with the ladybird beetle (*Hippodamia convergens*); unpublished report no. 347-107C, BVL no 2019794
Guideline:	EPA OPPTS 885.4340
GLP:	Yes
Material and methods:	
Test substance:	CARPOVIRUSINE; purity: 10^{13} CpGV/L
Test species:	Ladybird beetle (*Hippodamia convergens*), adult
Number of test animals:	3 replicates of 25 individuals per group
Treatments:	0, 55, 550 and 5500 ppm treatment group (corresponding to 5.5×10^8, 5.5×10^9 and 5.5×10^{10} GV/g diet); administered by honey.
Duration:	30 days
Test conditions:	Temperature: 26 - 28°C; Rel. Humidity: 69%; Photoperiod: 12-hours photoperiod
Deviations from guideline:	None
Endpoint:	Mortality, clinical signs of toxicity and abnormal behaviour
Observations:	The beetles were observed periodically in order to evaluate mortality, clinical signs of toxicity and abnormal behaviour. Observations were made approximately ¾ hour

and 2 hours after test initiation on Day 0, and then daily until Day 30.

Results:

At test termination on Day 30, mortality in the negative control group was 19%. Mortality in the 55, 550 and 5500 ppm treatment group was 32%, 17% and 20%, respectively, at test termination.

Immobile and/or lethargic beetles were observed from control and treatment groups during the test.
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	*Hippodamia convergens*
Toxicity of plant protection product	No signs of toxicity/infectivity/pathogenicity
	LC_{50} > 5500 ppm (5.5 x 10^{10} GV/g food)

Comments by the RMS (2019):

The study is acceptable.
The dietary LC_{50} value for ladybird beetles exposed to Carpovirusine for 30 days was determined to be greater than 5,500 ppm (5.5 x 10^{10} GV/g food), which was the highest concentration tested.
The NOEC was determined to be at least 5,500 ppm (5.5 x 10^{10} GV/g food).

Reference:	Hoxter K.A., Porch J.R., Krueger H.O. (1999b): Carpovirusine: A dietary pathogenicity and toxicity study with green lacewing larvae (*Chrysoperla carnea*); unpublished report no. 347-108, BVL no 3689731
Guideline:	EPA OPPTS 885.4340
GLP:	Yes
Material and methods:	
Test substance:	CARPOVIRUSINE; purity: 10^{13} CpGV/L
Test species:	Green lacewing larvae (*Chrysoperla carnea*); larva
Number of test animals:	30 per group
Treatments:	0, 55, 550 and 5500 ppm treatment group (corresponding to 5.5 × 10^8, 5.5 × 10^9 and 5.5 × 10^{10} GV/g diet); administered in a moth egg diet.
Duration:	10 days
Test conditions:	Temperature: 26 - 28°C; Rel. Humidity: 83%; Photoperiod: 12-hours photoperiod
Deviations from guideline:	None
Endpoint:	mortality, pupation of larvae, clinical signs of toxicity or abnormal behaviour
Observations:	The lacewing larvae were observed periodically in order to evaluate mortality, pupation of larvae, clinical signs of toxicity or abnormal behaviour. Observations were made approximately 2 hours after test ini-tiation on Day 0, and then daily until Day 10.

Results:

At test termination on Day 10, mortality in the negative control group was 30%. Mortality in the 55, 550 and 5500 ppm treatment group was 0%, 23% and 23%, respectively, at test termination.
At test termination on Day 10, pupation in the negative control group exceeded 20%. Pupation in the 55, 550 and 5500 ppm treatment group was 0%, 3% and 3%, respectively, at test termination.

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	*Chrysoperla carnea*
Toxicity of plant protection product	No signs of toxicity/infectivity/pathogenicity
	LC_{50} > 5500 ppm (5.5 x 10^{10} GV/g food)

Comments by the RMS (2019):

The study is acceptable.
The dietary LC_{50} value for green lacewing larvae (*Chrysoperla carnea*) exposed to CARPOVIRUSINE for 10 days was determined to be greater than 5500 ppm (5.5 × 10^{10} GV/g), which was the highest concentration tested. The NOEC was determined to be at least 5,500 ppm (5.5 x 10^{10} GV/g food).

Extended tests with the formulated product CARPOVIRUSINE were conducted in *Aphidius rhopalosiphi* and *Typhlodromus pyri* which are the standard sensitive species recommended to be used in ESCORT 2 Workshop of 2000 (Candolfi et al., 2001).

Reference:	Moll, M. (2006): Effects of Carpovirusine on the Parasitoid *Aphidius rhopalosiphi*, Extended Laboratory Study - Dose Response Test-; unpublished report no. 26192002, BVL no 3689735
Guideline:	- Mead-Briggs et al.2000: A laboratory test for evaluating the effects of plant protection products on the parasitic wasp, *Aphidius rhopalosiphi* (DeStephani-Perez) (Hymenoptera, Braconidae). - Current improvements by the ring-test group (Mead-Briggs et al. 2002)
GLP:	Yes
Material and methods:	
Test substance:	CARPOVIRUSINE; purity: 10^{13} CpGV/L
Test species:	*Aphidius rhopalosiphi*, female adults, not older than 48 hours
Number of test animals:	Exposure period: 5 females per replicate (6 replicates per treatment group); Post exposure period: 1 female per replicate (20 replicates per treatment group)
Treatments:	37 – 111 – 333 – 1000 – 3000 mL/ha; toxic standard: Perfekthion (content: 400 g Dimethoate/L) at 4 g a.s./ha in 400 L water/ha.
Duration:	48 h exposure period; 12-13 days test period including fecundity assessment
Test conditions:	Food: A solution of fructose (10%), in small test tubes (Ø approximately 1 cm) which will be connected with the exposure units at the beginning of the experiment; Temperature: 20 ± 2°C;Photoperiod: 16 h light : 8 h dark; Light intensity: 400 – 1170 lux (acclimatisation, exposure, parasitisation period), 12700 – 16200 lux (post-parasitisation period); Humidity: 60 - 90% (acclimatisation, exposure, parasitisation period), 60 – 63% (post-parasitisation period). Extended Laboratory Study - Dose Response Test: The product CARPOVIRUSINE was applied on barley seedlings at 5 doses ranging from 37 mL/ha to 3000 mL/ha. Additional barley seedlings were treated with water as control and dimethoate as toxic standard.
Deviations from guideline:	None
Endpoint:	mortality, fecundity, clinical signs of toxicity or abnormal behaviour
Observations:	- Mortality: mortality was recorded 2 – 24 – 48 hours after test initiation.

The number of parasitoids alive, affected, moribund and dead was rec-orded. Moribund parasitoids were counted as dead.

- Behaviour:

To determine whether residues of the test item were repel-lent to the wasps, observations on the position of the individual insects were made during the initial 3 hour after their release.

- Fecundity:

Number of aphids mummies were counted 10 - 11 days af-ter the 24 hour parasitisation period.

The fecundity assessment was performed where corrected mortality was ≤ 50%.

No fecundity testing was performed with the reference item.

Results:

A summary of endpoints is given in the table below.

Table B.9.4-1: Effects of CARPOVIRUSINE on *A. rhopalosiphi* after exposure for 48 hours on barley seeds

	Carpovirusine					**Control**	**Toxic standard**
Test item (mL product/ha)	**37.0**	**111**	**333**	**1000**	**3000**		
∑ wasps tested	30	30	30	30	30	30	30
∑ alive wasps	30	30	30	30	29	30	4
∑ affected wasps	0	0	0	0	0	0	0
∑ moribund wasps	0	0	0	0	0	0	0
∑ dead wasps	0	0	0	0	1	0	26
Mean mortality (%)	0.0	0.0	0.0	0.0	3.3 ± 8.2	0.0	86.7 ± 10.3
Corrected mortality (%)	0.0	0.0	0.0	0.0	3.3	-	86.7

Table B.9.4-2: Effects of CARPOVIRUSINE on reproductive capacity of female wasps

	Carpovirusine					**Control**	**Toxic standard**
Test item (mL product/ha)	**37.0**	**111**	**333**	**1000**	**3000**		
Parasitisation rate (mummies/female)	38.7 ± 18.7	42.6 ± 24.9	52.9 ± 22.2	51.4 ± 24.2	45.0 ± 28.6	56.4 ± 26.9	-
Reduction of beneficial capacity (%)	31.4	24.4	6.1	8.8	20.1	-	-

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} > 3000 mL/ha

Comments by the RMS (2019):

The study is acceptable.
According to the results obtained in this study, LR_{50} of CARPOVIRUSINE for adults of *Aphidius rhopalosiphi* under extended laboratory conditions is > 3000 mL product/ha.
There was no effect on reproduction up to and including 3000 mL CARPOVIRUSINE/ha compared to

the control.

Reference:	Rosenkranz, B. (2006): Effects of Carpovirusine on the Predatory Mite *Typhlodromus pyri*, Extended Laboratory Study -Dose Response Test-; unpublished report no. 26193062, BVL no 3689736
Guideline:	Blümel et al., 2000: Laboratory residual contact test with the predatory mite Typhlodro-mus pyri Sheuten (Acari: Phytoseiidae) for regulatory testing of plant protection prod-ucts. Oomen, 1988: Guideline for the evaluation of side-effects of pesticides on Phytoseiulus persimilis A.-H
GLP:	Yes
Material and methods:	
Test substance:	CARPOVIRUSINE; purity: 10^{13} CpGV/L
Test species:	*Typhlodromus pyri*, protonymphs, not older than 24 hours
Number of test animals:	10 per test unit (6 replicates per treatment group)
Treatments:	37 – 111 – 333 – 1000 – 3000 mL/ha; toxic standard: Perfekthion (content: 400 g Dimethoate/L) at 16 g a.s./ha in 400 L water/ha.
Duration:	7 days exposure period; reproductive performance was recorded at days 7 – 10 – 13 – 14 after test initiation
Test conditions:	Food: mixture of pine (*Pinus nigra*) and birch (*Betula* sp.) pollen (3:1) ad libitum on the day of the test start and on each assessment day except for the last one resp. at least every four days; Temperature: 25 - 26°C; Photoperiod: 16 h light : 8 h dark; Light intensity: 220 – 760 Lux; Humidity: 61% ± 81% Extended Laboratory Study - Dose Response Test: The product CARPOVIRUSINE was applied on leaf surfaces (bean plant, *Phaseolus vulgaris*); Additional leaves were treated with water as control and dimethoate as toxic standard.
Deviations from guideline:	None
Endpoint:	mortality, reproduction rate, clinical signs of toxicity or abnormal behaviour
Observations:	Mortality: The number of living, dead and escaped mites was counted at day 3 and at day 7 after test initiation. Further 3 assessments were carried out with a maximum interval of 3 days up to day 14 (inclusive). Dead mites were removed, escaped mites were considered as dead. Sex-ratio: The sex-ratio for reproduction testing at 7 day was 1 male: 5 females at a minimum. If at day 7 the sex-ratio was less than 1 male: 5 females, males originating from another replicate from the same treatment were added until an appropriate sex-ratio was reached. Reproduction: Number of eggs laid and number of live and dead juvenile stages per female counted at days 7 – 10 – 13 – 14 after test initiation in the con-trol and all treatment groups with a 7 day corrected mortality ‹ 50%.

Results:

A summary of endpoints is given in the table below.

Table B.9.4-3: Effects of CARPOVIRUSINE on *T. pyri*

Test item	CARPOVIRUSINE						
Test species	*T. pyri*						
Exposure	Detached primary bean leaves (*Phaseolus vulgaris*)						
Test formulation	Control (deionised water)	CARPOVIRUSINE (mL/ha)					Reference item Perfekthion[c] (mL/ha)
Application	200 L/ha	37.0	111	333	1000	3000	40
Mortality [%] (1 week after application)	18.3	8.3	28.3	13.3	25.0	26.7	70.0
Significance (Dunnett-test, α = 0.05)	-	n.s.	n.s.	n.s.	n.s.	n.s.	*
Corrected mortality [%][a]	-	-12.2	12.2	-6.1	8.2	10.3	63.3
LR_{50}	-	> 3000 mL/ha					
Reproduction rate (mean no. of eggs per female)	4.7	5.7	5.8	5.9	5.2	5.0	No reproduction evaluated
Significance (Dunnett-test, α = 0.05)	-	n.s.	n.s.	n.s.	n.s.	n.s.	
Effect on reproduction [%][b]	-	-21.3	-23.4	-25.5	-10.6	-6.4	

* significant compared to the control; n.s. not significant; - not applicable
a negative value means decreased mortality compared to the control
b negative value means increased reproduction compared to the control
c statistics with the reference substance was performed with Student t-test (α = 0.05)

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} > 3000 mL/ha

Comments by the RMS (2019):

The study is acceptable.
According to the results obtained in this study, LR_{50} of CARPOVIRUSINE on the predatory mite *Typhlodromus pyri* under extended laboratory conditions is > 3000 mL product/ha
There was no effect on reproduction up to and including 3000 mL CARPOVIRUSINE/ha compared to the control.

B.9.4.2 Risk assessment for arthropods other than bees

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

- High selectivity: *Cydia pomonella* Granulovirus (CpGV) is highly specific and only has an effect on very few species of the Tortricidae family (Lepidoptera).
- There are no major deviations from the GAP uses previously assessed in the DAR (2008) and the max. total rate per crop/season is identical.
- 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
Effects of the formulation CARPOVIRUSINE on non-target arthropods other than bees have been assessed for the first submission. Therefore, all relevant data were assessed in the EU review. Risk assessments for CARPOVIRUSINE 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 CARPOVIRUSINE to *non-target arthropods other than bees* was evaluated in extended 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 *non-target arthropods other than* bees indicate that CARPOVIRUSINE is 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.9.4-4: 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)	OECD Dossier, Doc M, IIIM, Sec. 6, Point 10.4 & 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)	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				EFSA Journal 2012;10(4):2655[7]

[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

	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	

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:

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

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

Table B.9.4-5: Exposure Assessment for the single application rate of CARPOVIRUSINE

Crop	**EC_{50} [L product/ha]**	**Single application rate [L product/ha]**	**MOS**
Pome fruits, stone fruits, walnut	> 3.00	1.00	3.00

MOS = Margin of safety

Table B.9.4-6: Exposure Assessment for the accumulated application rate of CARPOVIRUSINE

Crop	**EC_{50} [L product/ha]**	**Maximum application rate [L product/ha]**	**MOS**
Pome fruits, stone fruits, walnut	> 3.00	10.0	0.300

MOS = Margin of safety

A low margin of safety is derived for the exposure to non-target arthropods after the use of CARPOVIRUSINE after multiple applications according to GAP based on up to 10 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 546/2001, 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,

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

[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

(b) other biological properties,
(c) studies on toxicity, pathogenicity and infectivity to honeybees and other arthropods.

And in article 2.8.4.2[9], 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 546/2001, Part II, Uniform principles for evaluation and authorisation of plant protection products containing micro-organisms[10], 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 CpGV 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

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

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

B.9.5.1 Toxicity, infectiveness and pathogenicity in earthworms

Plant protection product

Reference:	Benech, B. (1996): Evaluation of the acute toxicity of Carpovirusine to earthworms (*Eisenia fetida*) using artificial soil substrate; unpublished report no. 96-64-003-BB, BVL no 3689740
Guideline:	ISO 1128-1
GLP:	Yes
Material and methods:	
Test substance:	CARPOVIRUSINE; purity: 6.7×10^{12} GV/L
Test species:	*Eisenia fetida andrei,* more than 2 months old
Number of test animals:	10 per group
Treatments:	0.1, 1.0, 10.0, 100 and 1000 mg/kg; negative control with water; positive control (chloroacetamid at 20 and 80 mg/kg)
Duration:	14 days exposure period
Test conditions:	Soil substrate: 10% sphagnum peat, 20% kaolinite clay, 70% fine sand, Food: Not specified; Temperature: 20°C ± 1.0°C; Photoperiod: 12 hour of light per day; Light intensity: 400 - 800 lux
Deviations from guideline:	None.
Endpoint:	Survival, body weight, signs of abnormal behaviour
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 occurred in the negative control over the test period.
Mortality was equal to 0% and 100% in the positive control group for 20 and 80 mg chloroacetamid/kg, respectively.
In the treatment groups, no mortality was observed except for the concentration of CARPOVIRUSINE of 1.0 mg/kg for which 10% of the earthworms were found dead at the end of the 14 days.
In the negative control mean weight remained almost unchanged.
In the positive control no variation occurred in the group exposed to 20.0 mg chloroacetamid/kg. As all earthworms were dead at the end of the test, biomass was not measured for the second positive control (80.0 mg/kg). Loss of biomass appeared in some CARPOVIRUSINE treatments but it seems to be non-related effect.

A summary of endpoints is given in the table below.

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

Test species	*Eisenia fetida andrei*
Toxicity of plant protection product	No signs of toxicity/infectivity/pathogenicity

Comments by the RMS (2019):

The study is acceptable.
The 14-day median lethal concentration (LC_{50}) of CARPOVIRUSINE in the earthworm was estimated to be > 1000 mg per kg of dry soil. The No-Observed Effect Concentration (NOEC) was established to be 1000 mg per kg of dry soil.

Reference:	Lührs, U. (2007a): Acute Toxicity (14 Days) of Carpovirusine to the Earthworm *Eisenia fetida* in Artificial Soil; unpublished report no. 26195021, BVL no 3689741
Guideline:	OECD 207, 1984 and ISO 11268-1, 1993
GLP:	Yes
Material and methods:	
Test substance:	CARPOVIRUSINE; purity: 1.0×10^{13} CpGV/L
Test species:	*Eisenia fetida* (Savigny 1826), ca. 11 months old, with clitellum
Number of test animals:	10 per group
Treatments:	1000 mg/kg (limit-Test); negative control with water
Duration:	14 days exposure period
Test conditions:	Food: None; Temperature: 20°C ± 2°C;Photoperiod: Continuous; Light intensity: 490 - 610 lux; Light intensity: 400 - 800 lux
Deviations from guideline:	None.
Endpoint:	Survival, body weight, signs of abnormal behaviour
Observations:	Mortality: The number of dead earthworms counted at days 7 and 14 after application. Behavioural abnormalities: Number of affected earthworms (lack of movement, rigidity) determined at days 7 and 14 after application. Mean body weight: Per test container determined at start and 14 days after application, using the same washing and weighing procedures as at test initiation.

Results:

Validity criteria of the study:
Control mortality: In this study the control mortality was 0% at day 14, and was therefore well within acceptable limits fixed at maximum 10%.
Control mean loss of biomass: The mean biomass in the controls increased by 2.4% after 14 days, and was therefore well within acceptable limits fixed at maximum 20%.

Mortality:
No mortality was observed within the 14 days of experimental time in any of the treatment groups.
According to these results the LC50 of CARPOVIRUSINE to *Eisenia fetida* after 14 days in artificial soil was estimated to be greater than 1000 mg test item/kg soil.
Behavioural Abnormalities: No behavioural effects were observed.

Body weights and behavioural abnormalities of *Eisenia fetida:*
Body weight changes: The body weight changes of the earthworms exposed to CARPOVIRUSINE at the concentration of 1000 mg/kg soil dry weight were not significantly different compared to the control (Student-t test, $\alpha = 0.05$).
Therefore, the Lowest-Observed-Effect-Concentration (LOEC) was determined to be greater than 1000 mg test item/kg soil and the No-Observed-Effect-Concentration (NOEC) was determined to be 1000 mg test item/kg soil.

A summary of endpoints is given in the table below.

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

Test species	*Eisenia fetida*
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.
According to the results of this study the LC_{50} and the Lowest-Observed-Effect-Concentration (LOEC) of CARPOVIRUSINE to earthworms (*Eisenia fetida*) was estimated to be greater than 1000 mg test item/kg soil. The No-Observed-Effect-Concentration (NOEC) was determined to be 1000 mg test item/kg soil.

Arysta LifeScience also conducted a further study for the determination of the effects of CARPOVIRUSINE on reproduction and growth of earthworms.

Reference:	Lührs, U. (2007b) Effects of Carpovirusine on Reproduction and Growth of Earthworms *Eisenia fetida* in Artificial Soil; unpublished report no. 26196022, BVL no 3689742
Guideline:	OECD 222, 2004 and ISO 11268-2, 1998
GLP:	Yes
Material and methods:	
Test substance:	CARPOVIRUSINE; purity: 1.0×10^{13} CpGV/L
Test species:	*Eisenia fetida* (Savigny 1826), 11 - 12 months old, with well-developed clitellum. Age range between test individuals not differing by more than 4 weeks.
Number of test animals:	10 per replicate; 4 replicates for the test item treatments, 8 replicates for the control
Treatments:	250, 500, 1000 mg/kg; negative control with water
Duration:	56 days exposure period
Test conditions:	Food: Fine ground cattle manure was used as food. 10 g/kg dry soil was mixed into the artificial soil 1 day before start of the study; 5 g/container was scattered on the soil surface at day 1 after application and was moistened with 5 - 6 g deionised water; 5 g/container (moistened with 5 - 6 g deionised water) was added each week for the first 4 weeks of the experiment, when the food of the previous week was almost consumed. If the food was not quite fully consumed, the added amount of food was adjusted to the visually estimated consumption. 4 weeks after application, the food was mixed by hand into the substrate following removal of the adult worms. Temperature: Acclimatisation : 20°C - 21°C, Exposure : 19°C – 21°C;Photoperiod: 16 h light : 8 h dark; Light intensity: 480-670 lux
Deviations from guideline:	None.
Endpoint:	Survival, body weight, feeding activity, reproduction, signs of abnormal behaviour
Observations:	Mortality, body weight and feeding activity: The number of dead adult earthworms, of sublethally affected worms, body weights and the amount of food added to each test container (which roughly reflects the amount of food eaten) were listed for each treatment. Reproduction : The number of offspring was listed for each container

Results:

Validity criteria of the study:
Control mortality: In this study the control mortality was 0%, and was therefore well within acceptable limits fixed at maximum 10%.
Reproduction of Control: The number of worms per replicate was 254 to 435 and was therefore well above the acceptable limit of minimum 30 worms per replicate.
Coefficient of Variation of Reproduction in Control was 19.5% and therefore well below the acceptable limit of maximum 30%

Positive control:
In a separate study (study code 21343022) the reference item Brabant Carbendazim Flowable showed statistically significant effects on reproduction at a concentration of 0.5 mg carbendazim/kg artificial soil (dry weight); the EC_{50} for reproduction was calculated as 1.14 mg carbendazim/kg soil dry weight

Mortality:
No mortality was observed in any of the treatment groups.
No behavioural abnormalities were observed and all worms did burrow into the soil within 15 min after introduction.

Body weights of the adult earthworms:
The earthworm body weight changes in the test item treated groups were not significantly different compared to the control (Dunnett test, $\alpha = 0.05$, two-sided).

Reproductive Assessment:
Reproduction (mean number of offspring worms per container): The reproduction rates were not significantly different compared to the control in any test item treated groups (Dunnett test, $\alpha = 0.05$, one-sided smaller).

Feeding Activity and Behavioural Abnormalities:
Feeding activity: food was consumed in all treatment groups. The results show that the turnover of biomass of those earthworms exposed to the three different rates of the test item was comparable to the control.
The method used here only approximately assesses food consumption of *Eisenia fetida*, because any food remaining cannot be weighed again. However, it reflects roughly to what extent the ecological role of earthworms in decomposing organic material was affected.

A summary of endpoints is given in the table below.

Table B.9.5-1: Summary of the effects of Carpovirusine on earthworms in a 56-day reproduction study

	Control	**Carpovirusine [mg/kg soil dry weight**		
	--	**250**	**500**	**1000**
Mean mortality (day 28) [%]	0.0	0.0	0.0	0.0
Mean weight change (day 28) [%]	41.2	36.4	35.1	34.6
Mean number of juveniles (day 56)	307	339	313	297
Reproduction in [%] of control	--	110.6	102.0	96.7

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

Test species	*Eisenia fetida*
Toxicity of plant protection product	No signs of toxicity/infectivity/pathogenicity

	NOEC ≥ 1000 mg/kg soil dw

Comments by the RMS (2019):

The study is acceptable.
In this study the no-observed-effect-concentration (NOEC) of Carpovirusine for mortality, growth, reproduction and feeding activity of the earthworm *Eisenia fetida* was 1000 mg test item/kg soil dry weight, i.e. the highest concentration tested.

B.9.5.2 Risk assessment for earthworms

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

- High selectivity: *Cydia pomonella* Granulovirus (CpGV) is highly specific and only has an effect on very few species of the Tortricidae family (Lepidoptera).
- There are no major deviations from the GAP uses previously assessed in the DAR (2008) and the max. total rate per crop/season is identical.
- 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

Effects of the formulation CARPOVIRUSINE on earthworms have been assessed for the first submission. Therefore, all relevant data were assessed in the EU review. Risk assessments for CARPOVIRUSINE 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 CARPOVIRUSINE 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[11]).
All available data for earthworms demonstrate that CpGV as any other baculovirus and the formulated product CARPOVIRUSINE 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.9.5-2: Summary of the studies on effects to earthworms

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

[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
[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

	Eisenia fetida	56-day, reproduction **1000 mg product/kg soil (dw)***	
GRANUPOM (as Granulosevirus CpGV SC; 2.2×10^{13} GV/L)	*Eisenia fetida*	14-day, acute 1000 mg product/kg soil (dw) (1.67×10^{10} GV/kg soil (dw))*	EFSA Journal 2012;10(4):2655[12]
VIRGO (2.0×10^{13} GV/L)	*Eisenia fetida*	14-day, acute 1000 mg product/kg soil (dw) (1.61×10^{10} GV/kg soil (dw))*	EFSA Journal 2012;10(4):2655[12]

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

Predicted environmental population density in soil

In order to perform a risk assessment for non-target organisms the actual population of *Cydia pomonella* Granulovirus (CpGV) is calculated for soil, based on the maximum accumulated application rate of 10 L product/ha in pome fruits, stone fruits and walnut upon foliar application, assuming 10 treatments of 1.0 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 1.0×10^{13} GV/L product has been considered.

Assumptions:

- Application rate CARPOVIRUSINE: 1 L product/ha (equivalent to 1.0×10^{13} GV/ha)
- Accumulated application rate (up to 10 treatments): 10 L product/ha, equivalent to 1.0×10^{14} 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.9.5-3: Calculation of the predicted environmental density of CARPOVIRUSINE and CpGV in soil (PED_{soil}) after 10 applications at 1.0 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]***
10.472	1047.2	5.00	1.5	13.96
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]**
1.0×10^{14}	1.0×10^{10}	5.00	1.5	1.33×10^8

* calculated with a density of CARPOVIRUSINE of 1.0472 g/cm³

According to the PED_{soil} calculation the expected initial density is 13.96 mg product/kg dry soil, corresponding to 1.33×10^8 GV/kg dry soil.

Risk Assessment
The acute toxicity of CARPOVIRUSINE against *Eisenia fetida* has been investigated in two 14-day acute and one 56-day reproduction laboratory studies. The LC_{50} was determined to be above 1000 mg product/kg soil dw in all tests. 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 1.0 L product/ha in pome fruits, stone fruits and walnut. The predicted environmental density in soil (PED_{soil}) was calculated as 1.33×10^8 GV/kg soil dw (corresponding to 13.96 mg product/kg soil dw) for multiple application in pome fruits, stone 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:

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

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

Table B.9.5-4: MOS calculation for earthworms

Use pattern	Test organism	LC_{50} [mg product/kg soil dw]	PED_{soil} [mg product/kg soil dw]	MOS
10 × 1 L product/ha in pome fruits, stone fruits and walnut	*Eisenia fetida*	> 1000	13.96	> 71.6

MOS = Margin of safety

The calculated MOS value is high, indicating an acceptable acute risk to earthworms after application of CARPOVIRUSINE 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

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

Reference:	Servajean, E. (2001): Laboratory assessment of the side-effects of Carpovirusine on the soil micro-organisms; unpublished report no. 00-64-004-ES, BVL no3689622
Guideline:	SETAC Guidelines: procedures for assessing the environmental fate and ecotoxicity of pesticides, 1995
	OECD Guideline for testing of chemicals – Soil micro-organisms: nitrogen mineralization test, 1996.
	OECD Guideline for testing of chemicals – Soil micro-organisms: Carbon mineralization test, 1996.

GLP:	Yes
Material and methods:	
Test substance:	CARPOVIRUSINE; purity: 1.0×10^{13} CpGV/L
Reference substance:	Benlate (50% benomyl)
Treatments:	Control (deionised water) Carpovirusine: 2.7×10^{7} CpGV/L Reference: Benlate: 1 kg/ha as positive control
Duration:	28 days
Test conditions:	A sandy soil and a soil collected in orchard were used, respectively. Temperature: 20°C ± 2°C; Photoperiod: darkness; Moisture content: 45% of the WHC_{max}
Deviations from guideline:	None.
Endpoint:	Nitrogen turnover, short-term respiration
Observations:	Observations: Nitrogen turnover after addition of ground lucerne and on short-term respiration after addition of glucose on day 0, 14, 28

Results:

In the treatment group, the deviation of the nitrate contents of soil type 1 and soil type 2 was less than 25% from the control group within the 28 days incubation period. Therefore, the impact of CARPOVIRUSINE on soil nitrogen turnover is considered as negligible even at a dosage of 2.7×10^{7} granules CpGV/kg dry soil. Also the short term respiration of the soil microflora was not significantly different from the control over a 28 d period at a dosage of 2.7×10^{7} granules CpGV/kg dry soil. The reference substance inhibited the content of nitrogen within the 28 days incubation in both soil types significantly between 7 and 48%. Short-term respiration was not affected by the reference substance.

A summary of the findings is given in the tables below.

Table B.9.6-1: Parameters of the soils

Soil Type	Sandy soil	orchard soil
Soil Origin	Argelouse, France	St Nicolas de la Grave, France
pH	7.1	8.1
Organic carbon (%)	1.20	1.49
Organic matter	2.07	2.57
Total N (mg/kg dry mass)	450	1460
Maximum water holding capacity (mL H_2O/100 g soil dry mass)	31.4	57.5

Table B.9.6-2: Mean concentration of nitrates (N-NO_3 mg/kg dry weight) in sandy soil

Treatment	**Unamended soils**			**Amended soils**		
	Day 0	**Day 14**	**Day 28**	**Day 0**	**Day 14**	**Day 28**
Control	36.40 ± 3.41	31.74 ± 2.92	36.29 ± 2.94	6.58 ± 1.00	59.35 ± 2.43	85.48 ± 2.18
Carpovirusine	40.95 ± 3.45	36.56 ± 4.45	47.88 ± 4.71*	8.08 ± 1.02	62.53 ± 10.70	83.98 ± 4.82
Benlate	31.76 ± 0.90	29.18 ± 1.89	32.73 ± 1.99	7.56 ± 1.82	58.74 ± 4.19	79.54 ± 3.30*

*: statistically different from the control soil

Table B.9.6-3: Mean concentration of nitrates (N-NO_3 mg/kg dry weight) in soil of orchard

Treatment	Unamended soils			Amended soils		
	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28
Control	8.84 ± 2.50	7.33 ± 0.89	16.08 ± 1.20	1.29 ± 1.30	5.88 ± 0.92	11.19 ± 1.09
Carpovirusine	6.86 ± 3.33	8.70 ± 2.18	14.25 ± 0.86	1.75 ± 1.32	3.85 ± 0.31	9.87 ± 2.55
Benlate	6.56 ± 0.60	8.54 ± 2.15	13.19 ± 1.32*	0.54 ± 0.02	4.55 ± 0.30	5.81 ± 0.70*

*: statistically different from the control soil

Table B.9.6-4: Mean oxygen consumption (mg/kg soil/h) throughout a 12-hour glucose induced respiration in sandy soil

Treatment	Unamended soils			Amended soils		
	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28
Control	54.03 ± 1.42	29.47 ± 2.13	13.51 ± 1.23	53.62 ± 1.88	59.76 ± 0.71	44.21 ± 3.25
Carpovirusine	45.03 ± 1.42	30.29 ± 2.56	14.74 ± 3.25	50.76 ± 3.09	56.08 ± 1.88*	46.66 ± 4.43
Benlate	47.07 ± 0.71	30.29 ± 0.71	13.10 ± 1.42	52.80± 1.23	57.31 ± 0.71*	46.66 ± 2.13

*: statistically different from the control soil

Table B.9.6-5: Mean oxygen consumption (mg/kg soil/h) throughout a 12-hour glucose induced respiration in soil of orchard

Treatment	Unamended soils			Amended soils		
	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28
Control	36.02 ± 2.56	26.61 ± 3.75	30.70 ± 3.68	45.44 ± 3.68	37.25 ± 1.88	29.06 ± 1.88
Carpovirusine	37.25 ± 1.88	31.52 ± 3.95	34.79 ± 3.09	46.66 ± 1.23	40.93 ± 1.42*	26.61 ± 1.42
Benlate	36.84 ± 1.23	28.65 ± 4.31	29.88 ± 2.84	45.44 ± 2.13	39.30 ± 3.47	28.24 ± 2.46

*: statistically different from the control soil

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 (< 25% deviation) even at a dosage of 2.7×10^7 CpGV/kg dry soil, corresponding to 2×10^{13} granules CpGV/ha (assuming a uniform distribution to a soil depth of 5 cm).

B.9.6.1 Impact on non-target soil micro-organisms

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

B.9.6.2 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) and the max. total rate per crop/season is identical.
- 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 micro-organisms is provided below for illustrative

purposes.

Effects on soil micro-organisms

Effects of the formulation CARPOVIRUSINE on soil micro-organisms have been assessed for the first submission. Therefore, all relevant data were assessed in the EU review. Risk assessments for CARPOVIRUSINE 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 CARPOVIRUSINE to soil micro-organisms was evaluated (please refer to the OECD Dossier, Doc IIIM, Section 6, Point IIIM 10.6 and EFSA Journal 2012;10(4):2655[13]).
All available data demonstrate that CpGV as any other baculovirus and the formulated product CARPOVIRUSINE are does not have any effect on soil microorganisms.
The EU agreed endpoints are summarised in the following table.

Table B.9.6-6: 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 × 1013 GV/ha)**	OECD Dossier, Doc M, IIIM, Sec. 6, Point 10.6 & EFSA Journal 2012;10(4):2655[13]
	N		
GRANUPOM (2.2×10^{13} GV/L)	C	1.33×10^{8} GV/kg soil (dw) (corresponding to 1.0×10^{14} GV/ha)	EFSA Journal 2012;10(4):2655[13]
	N		
VIRGO (2.0×10^{13} GV/L)	C	1.33×10^{8} GV/kg soil (dw) (corresponding to 1.0×10^{14} GV/ha)	EFSA Journal 2012;10(4):2655[13]
	N		
VIRGO (2.0×10^{13} GV/L)	C	2.0×10^{8} GV/kg soil (dw) (corresponding to 1.5×10^{14} GV/ha)	EFSA Journal 2012;10(4):2655[13]
	N		

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

Risk assessment
The toxicity of CARPOVIRUSINE 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 1.0 L product/ha in pome fruits, stone fruits and walnut. The predicted environmental density in soil (PED_{soil}) was calculated as 1.33×10^{8} GV/kg soil dw (corresponding to 13.96 mg product/kg soil dw) for multiple application in pome fruits, stone fruits and walnut, assuming a worst-case scenario that no interception and no degradation occurs between applications.

Table B.9.6-7: Risk assessment for soil micro-organisms

Use pattern	Test organism	PED_{soil} [GV/kg soil (dw)]	Endpoint [GV/kg soil (dw)]

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

10 × 1 L product/ha in pome fruits, stone fruits and walnut	Soil microorganism	1.33×10^8	2.7×10^7

Cydia pomonella Granulovirus (CpGV) had no significant effect on soil functional parameters nitrogen conversion and carbon transformation at 2.7×10^7 GV/kg soil (dw), corresponding to 2×10^{13} GV/ha. Due to the absence of adverse effects observed in the laboratory study with CARPOVIRUSINE and endpoints up to 2.0×10^8 GV/kg soil (dw) obtained with comparable products, it can be assumed that GAP directed use of CARPOVIRUSINE 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 have been conducted with CARPOVIRUSINE.

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	N		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	no	not protected	PKA	Y KIII M 10.3
KMP 10.1	[redacted]	1993	CARPOVIRUSINE: AN AVIAN ORAL PATHOGENICITY AND TOXICITY STUDY IN THE NORTHERN BOBWHITE Arysta LifeScience S.A.S., 347-105 [redacted] GLP: yes Published: no 3689620	yes	no	not protected	ALS	Y KIIIM 10.1
KMP 10.2	[redacted]	1994a	TEST TO EVALUATE ACUTE TOXICITY (96 HOURS) IN FRESHWATER FISH (BRACHYDANIO RERIO) USING A STATIC METHOD Arysta LifeScience S.A.S., E150 Natural Plant Protection, Pau GLP: yes Published: no 3689633	yes	no	not protected	ALS	Y KIIIM 10.2
KMP 10.2	[redacted]	1994b	TEST TO EVALUATE ACUTE TOXICITY (48 HOURS) IN DAPHNIA Arysta LifeScience S.A.S., E151 Natural Plant Protection, Pau GLP: yes Published: no 3689641	no	no	not protected	ALS	Y KIIIM 10.2

KMP 10.2	Pawlowski, S., Wydra, V., Vinken, R.	2007	TOXICITY OF CARPOVIRUSINE TO PSEUDO-KIRCHNERIELLA SUBCAPITANA IN AN ALGAL GROWTH INHIBITION TEST FINAL REPORT Arysta LifeScience S.A.S., 26191210 Institut für Biologische Analytik, Rossdorf Germany GLP: yes Published: no 3689706	no	no	not protected	ALS	Y KIIIM 10.2
KMP 10.2	Jehle, J., Matt-Schmid, A.	2006	ANALYTICAL PHASE REPORT: TOXICITY OF CARPOVIRUSINE TO PSEUDOKIRCHNERIELLA SUBCAPITATA IN AN ALGAL GROTWH INHIBITION TEST Arysta LifeScience S.A.S., ARY05, 26191210 Dienstleistungszentrum Ländlicher Raum, Neustadt an der Weinstraße GLP: yes Published: no 3689555	no	no	not protected	ALS	Y KIIIM 10.2
KMP 10.4	Hoxter, K.A., Porch, J.R., Krueger, H.O.	1999a	CARPOVIRUSINE: A DIETARY PATHOGENICITY AND TOXICITY STUDY WITH THE LADYBIRD BEETLE (HIPPODAMIA CONVERGENS) Arysta LifeScience S.A.S., 347-107C Wildlife International Ldt., Easton, MD, United States GLP: yes Published: no 2019794	no	no	not protected	ALS	Y KIIIM 10.4
KMP 10.4	Hoxter, K.A., Porch, J.R., Krueger, H.O.	1999b	CARPOVIRUSINE: A DIETARY PATHOGENICITY AND TOXICITY STUDY WITH GREEN LACEWING LARVAE (CHRYSOPERLA CARNEA) Arysta LifeScience S.A.S., 347-108 Wildlife International Ldt., Easton, MD, United States GLP: yes Published: no 3689731	no	no	not protected	ALS	Y KIIIM 10.4
KMP 10.4	Moll, M.	2006	EFFECTS OF CARPOVIRUSINE ON THE PARASITOID APHIDIUS RHOPALOSIPHI, EXTENDEND LABORATORY STUDY - DOSE RESPONSE TEST- Arysta LifeScience S.A.S., 26192002 Institut für Biologische Analytik, Rossdorf Germany GLP: yes	no	no	not protected	ALS	Y KIIIM 10.4

			Published: no 3689735					
KMP 10.4	Rosenkranz, B.	2006	EFFECTS OF CARPOVIRUSINE ON THE PREDATORY MITE TYPHLODROMUS PYRI, EXTENDED LABORATORY STUDY -DOSE RESPONSE TEST- Arysta LifeScience S.A.S., 26193062 Institut für Biologische Analytik, Rossdorf Germany GLP: yes Published: no 3689736	no	no	not protected	ALS	Y KIIIM 10.4
KMP 10.5	Benech, B.	1996	EVALUATION OF THE ACUTE TOXICITY OF CARPOVIRUSINE TO EARTHWORMS (EISENIA FETIDA) USING ARTIFICIAL SOIL SUBSTRATE Arysta LifeScience S.A.S., 96-64-003-BB Envirotests, S. A. Pau, France GLP: yes Published: no 3689740	no	no	not protected	ALS	Y KIIIM 10.5
KMP 10.5	Lührs, U.	2007a	ACUTE TOXICITY (14 DAYS) OF CARPOVIRUSINE TO THE EARTHWORM EISENIA FETIDA IN ARTIFICIAL SOIL Arysta LifeScience S.A.S., 26195021 Institut für Biologische Analytik, Rossdorf Germany GLP: yes Published: no 3689741	no	no	not protected	ALS	Y KIIIM 10.5
KMP 10.5	Lührs, U.	2007b	EFFECTS OF CARPOVIRUSINE ON REPRODUCTION AND GROWTH OF EARTHWORMS EISENIA FETIDA IN ARTIFICIAL SOIL Arysta LifeScience S.A.S., 26196022 Institut für Biologische Analytik, Rossdorf Germany GLP: yes Published: no 3689742	no	no	not protected	ALS	Y KIIIM 10.5
KMP 10.6	Servajean, E.	2001	LABORATORY ASSESSMENT OF THE SIDE-EFFECTS OF CARPOVIRUSINE ON THE SOIL MICRO-ORGANISMS Arysta LifeScience S.A.S., 00-64-004-ES Envirotests, S. A. Pau, France	no	no	not protected	ALS	Y KIIIM 10.6

			GLP: yes Published: no 3689622					