Renewal Assessment Report

Cydia pomonella GV

Virgo

Volume 3 – B.6 Effects on human health

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Rapporteur Member State: Germany

Co-Rapporteur Member State: The Netherlands

Version history

When	What
16 October 2020	First version submitted to EFSA

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

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B Summary, evaluation and assessment of the data and information

B.6 Effects on human health

Virgo is one of four representative formulations for the renewal of approval of *Cydia pomonella* Granulovirus (CpGV). Virgo is formulated as suspension concentrate containing 2 x 10^{13} granules of CpGV (Mexican Isolate) per litre.

A literature research was conducted by the notifier and the RMS (see Vol. 3 CA B.6). No study was identified to be relevant for this chapter.

Virgo was already evaluated for the first approval of the active substance. The formulation has not been changed since then. No new studies were submitted. Instead, the old studies are reported again and re-evaluated by the RMS according to current scientific criteria and guidelines.

A summary of the toxicological evaluation for Carpovirusine is presented in Table B.6.1-1. The individual studies are described by the RMS in detail under B.6.1 to B.6.6.

Studies on the infectivity, toxicity and specificity of baculoviruses in general (Gröner, 1986, TOX2003-1179; Gröner et al., 1978, TOX2003-1154; Krieg, 1976, BWS2003-90) are discussed in the toxicology section on CpGV.

Table B.6.1-1: Summary of evaluation of the studies on acute toxicity including irritancy and skin sensitisation for Virgo

Type of test, model system (Guideline)	Result	Acceptability	Classification (acc. to the criteria in Reg. 1272/2008)	Reference
LD_{50} oral, rat (OECD 423)	> 2000 mg/kg bw	Yes	-	[redacted] 2005; TOX2006-1692
LD_{50} dermal, rat (OECD 402)	> 2000 mg/kg bw	Yes	-	[redacted] 2005; TOX2006-1058
LC_{50} inhalation, rat	> 5.10 mg/L air	Yes	-	[redacted] 2005; TOX2006-1054
Skin irritation, rabbit (OECD 404)	Non irritant	Yes	-	[redacted] 2005; TOX2006-1059
Eye irritation, rabbit (OECD 405)	Non irritant	Yes	-	[redacted] 2005; TOX2006-1060
Skin sensitisation, guinea pig (M&K) (OECD 406)	Non-sensitising	Yes	-	[redacted] 2005; TOX2006-1050

Virgo is of low toxicity by the oral, dermal and inhalation route. It is neither irritating to the skin nor to the eye and does not meet the criteria for classification as skin sensitiser. Due to a potential risk for sensitisation by micro-organisms including viruses (that cannot be ruled out by a negative result in a standard test considered to be not appropriate for testing microorganisms) the product has to be labelled with the phrase: "Contains *Cydia pomonella* Granulovirus. Micro-organisms may have the potential to provoke sensitizing reactions."

B.6.1 Basic acute toxicity studies

B.6.1.1 Acute oral toxicity

Study evaluated in the original monograph of *Cydia pomonella* Granulovirus (December, 2007, ASB2010-10675) and re-evaluated by RMS in 2018:

Reference:	KMP 7.1.1
Report	Acute oral toxicity Study of Virgo in CD Rats [redacted]2005, Report No. 18974/05, TOX2006-1692
Guideline(s):	OECD Guideline 423 (Acute Oral toxicity - Acute Toxic Class Method) EU Method B.1 tris (Acute Oral Toxicity - Acute Toxic Class Method)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test material (Lot/Batch No.)	Virgo (Batch No. VR310910)
Species	Rat, CD / Crl: CD(SD)
No. of animals (group size)	3 rats/sex
Dose(s)	2000 mg/kg bw
Exposure	Once by gavage
Vehicle/Dilution	None
Post exposure observation period	14 days
Remarks	None

Results and discussions

Table B.6.1-1: Results of acute oral toxicity study in rats of Virgo

Dose (mg/kg bw)	Toxicological results*	Duration of signs	Time of death	LD_{50} (mg/kg bw) (14 days)
Male rats				
2000	0/0/3	-	-	> 2000
Female rats				
2000	0/0/3	-	-	> 2000

* Number of animals which died/number of animals with clinical signs/number of animals used

Table B.6.1-2: Summary of findings of acute oral toxicity study in rats of Virgo

Mortality:	No mortality occurred.
Clinical signs:	No clinical signs of toxicity were observed. -
Body weight:	Body weight gain was considered to be normal.
Macroscopic examination:	The necropsies performed at the end of the study revealed no apparent findings.

Conclusion

Under the experimental conditions, the oral LD_{50} of Virgo is higher than 2000 mg/kg bw in rats. Thus, no classification is required according to Regulation (EC) No. 1272/2008.

Conclusion by the RMS (2019):

The study is still considered to be acceptable.

B.6.1.2 Acute inhalation toxicity

Study evaluated in the original monograph of *Cydia pomonella* Granulovirus (December, 2007, ASB2010-10675) and re-evaluated by RMS in 2018:

Reference:	OECD KIIIM1 7.1.3
Report	Acute inhalation toxicity study of Virgo in rats [redacted] 2005, Report No. 19016/05, TOX2006-1054
Guideline(s):	OECD Guideline 403 (Acute Inhalation Toxicity) EU Method B.2 (Acute Toxicity (Inhalation))
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test material (Lot/Batch No.)	Virgo (Batch No. VR310910)
Species	Rat, CD / Crl: CD(SD)
No. of animals (group size)	5 rats/sex
Concentration(s)	5.10 mg/L air
Exposure	4 hours (nose only)
Vehicle/Dilution	None
Post exposure observation period	14 days
Remarks	None

Results and discussions

Table B.6.1-3: Concentration(s) and exposure conditions

Nominal conc. (mg/L air)	Actual conc. (mg/L air)	MMAD* (µm)	GSD** (µm)
78.3	5.10	2.561	3.782

* MMAD = Mass Median Aerodynamic Diameter
** GSD = Geometric Standard Deviation

Table B.6.1-4: Results of acute inhalation toxicity study in rats of Virgo

Concentration (mg/L air)	Toxicological results*	Duration of signs	Time of death	LC_{50} (mg/L air) (14 days)
Male rats				

Concentration (mg/L air)	Toxicological results*	Duration of signs	Time of death	LC_{50} (mg/L air) (14 days)
5.10	0/0/5	-	-	> 5.10
Female rats				
5.10	0/0/5	-	-	> 5.10

* Number of animals which died/number of animals with clinical signs/number of animals used

Table B.6.1-5: Summary of findings of acute inhalation toxicity study in rats of Virgo

Mortality:	No mortality occurred.
Clinical signs:	No clinical signs of toxicity were observed. (If yes, describe kind of signs)
Body weight:	Body weight gain was considered to be normal.
Macroscopic examination:	The necropsies performed at the end of the study revealed no apparent findings.

Conclusion

Under the experimental conditions, the inhalation LC_{50} of Virgo is higher than 5.10 mg/L air in rats. Thus, no classification is required according to Regulation (EC) No. 1272/2008.

Conclusion by the RMS (2019):

The study is still considered to be acceptable.

B.6.1.3 Acute percutaneous toxicity

Study evaluated in the original monograph of *Cydia pomonella* Granulovirus (December, 2007, ASB2010-10675) and re-evaluated by RMS in 2018:

Reference: OECD KIIIM1 7.1.2

Report: Acute dermal toxicity study of Virgo in CD rats [redacted] 2005, Report No. 18975/05, TOX2006-1058

Guideline(s): OECD Guideline 402 (Acute Dermal Toxicity)
EU Method B.3 (Acute Toxicity (Dermal))

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

Test material (Lot/Batch No.)	Virgo (Batch No. VR310910)
Species	Rat, CD / Crl: CD(SD)
No. of animals (group size)	5 rats/sex
Dose(s)	2000 mg/kg bw
Exposure	24 hours (dermal, semi-occlusive)
Vehicle/Dilution	None
Post exposure observation period	14 days

Remarks	None

Results and discussions

Table B.6.1-6: Results of acute dermal toxicity study in rats of Virgo

Dose (mg/kg bw)	Toxicological results *	Duration of signs	Time of death	LD_{50} (mg/kg bw) (14 days)
Male rats				
2000	0/0/5	-	-	> 2000
Female rats				
2000	0/0/5	-	-	> 2000

* Number of animals which died/number of animals with clinical signs/number of animals used

Table B.6.1-7: Summary of findings of acute dermal toxicity study in rats of Virgo

Mortality:	No mortality occurred.
Clinical signs:	No clinical signs of toxicity were observed.
Body weight:	Body weight gain was considered to be normal.
Macroscopic examination:	The necropsies performed at the end of the study revealed no apparent findings.

Conclusion

Under the experimental conditions, the dermal LD_{50} of Virgo is higher than 2000 mg/kg bw in rats. Thus, no classification is required according to Regulation (EC) No. 1272/2008.

Conclusion by the RMS (2019):

The study is still considered to be acceptable.

B.6.2 Additional acute toxicity studies

B.6.2.1 Skin irritation

Study evaluated in the original monograph of *Cydia pomonella* Granulovirus (December, 2007, ASB2010-10675) and re-evaluated by RMS in 2018:

Reference:	OECD KIIIM1 7.1.4
Report	Acute dermal irritation/corrosion test (Patch test) of Virgo in rabbits [redacted] 2005, Report No. 18976/05, TOX2006-1059
Guideline(s):	EU Method B.4 (Acute Toxicity: Dermal Irritation / Corrosion) OECD Guideline 404 (Acute Dermal Irritation / Corrosion)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test material (Lot/Batch No.)	Virgo (Batch No. VR310910)
Species	Rabbit, Himalayan
No. of animals (group size)	3 males
Initial test using one animal	Yes
Exposure	0.5 mL (4 hours, semi-occlusive)
Vehicle/Dilution	None
Post exposure observation period	3 days
Remarks	None

Results and discussions

Table B.6.2-1: Skin irritation of Virgo

Animal No.		Scores after treatment*				Mean scores (24-72 h)	Reversible (day)
		1 h	24 h	48 h	72 h		
1	Erythema Oedema	0# 0	0# 0	0# 0	0 0	0 0	- -
2	Erythema Oedema	0# 0	0# 0	0 0	0 0	0 0	- -
3	Erythema Oedema	0# 0	0# 0	0 0	0 0	0 0	- -

* scores in the range of 0 to 4
\# blue discolouration of the application site

Clinical signs:	No clinical signs of toxicity were observed.

Conclusion

Under the experimental conditions, Virgo is not a skin irritant. Thus, no classification is required according to Regulation (EC) No. 1272/2008.

Conclusion by the RMS (2019):

The study is still considered to be acceptable.

B.6.2.2 Eye irritation

Study evaluated in the original monograph of *Cydia pomonella* Granulovirus (December, 2007, ASB2010-10675) and re-evaluated by RMS in 2018:

Reference: OECD KIIIM1 7.1.5

Report: Acute eye irritation/corrosion test of Virgo in rabbits [redacted] 2005, Report No. 18977/05, TOX2006-1060

Guideline(s): OECD Guideline 405 (Acute Eye Irritation / Corrosion)
EU Method B.5 (Acute Toxicity: Eye Irritation / Corrosion)

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

Test material (Lot/Batch No.)	Virgo (Batch No. VR310910)
Species	Rabbit, Himalayan
No. of animals (group size)	3 males
Initial test using one animal	Yes/No
Exposure	0.1 mL (single instillation in conjunctival sac)
Irrigation (time point)	Yes/No
Vehicle/Dilution	None
Post exposure observation period	14 days
Remarks	None

Results and discussions

Table B.6.2-2: Eye irritation of Virgo

Animal No.		Scores after treatment*				Mean scores (24-72 h)	Reversible (day)
		1 h	24 h	48 h	72 h		
1	Corneal opacity	0	0	0	0	0	-
	Iritis	0	0	0	0	0	-
	Redness conjunctivae	0	0	0	0	0	-
	Chemosis conjunctivae	0	0	0	0	0	-
2	Corneal opacity	0	0	0	0	0	-
	Iritis	0	0	0	0	0	-
	Redness conjunctivae	0	0	0	0	0	-
	Chemosis conjunctivae	0	0	0	0	0	-
3	Corneal opacity	0	0	0	0	0	-
	Iritis	0	0	0	0	0	-
	Redness conjunctivae	0	0	0	0	0	-
	Chemosis conjunctivae	0	0	0	0	0	-

* Scores in the range of 0 to 4 for cornea opacity and chemosis, 0 to 3 for redness of conjunctivae and 0 to 2 for iritis

Clinical signs:	No clinical signs of toxicity were observed.

Conclusion

Under the experimental conditions, Virgo is not an eye irritant. Thus, no classification is required according to Regulation (EC) No. 1272/2008.

Conclusion by the RMS (2019):

The study is still considered to be acceptable.

B.6.2.3 Skin sensitisation

Study evaluated in the original monograph of *Cydia pomonella* Granulovirus (December, 2007, ASB2010-10675) and re-evaluated by RMS in 2018:

Reference:	KMP 7.2.3
Report	Examination of Virgo in the skin sensitisation test in Guinea pigs according to Magnusson and Kligman (Maximisation test); [redacted] 2005, Report No. 18978/05, TOX2006-1050
Guideline(s):	EU Method B.6 (Skin Sensitisation: GPMT) OECD Guideline 406 (Skin Sensitisation: GPMT)
Deviations:	Test item was only tested in a concentration of 50% for topical induction and challenge although no skin reactions were observed at this concentration in the pre-test
GLP:	Yes

Materials and methods

Test material (Lot/Batch No.)	Virgo (Batch No. VR310910)
Species	Guinea pig, Dunkin-Hartley
No. of animals (group size)	Test substance group: 10 female guinea pigs Vehicle control goup: 5 female guinea pigs
Range finding:	Yes
Exposure (concentration(s), no. of applications)	Intradermal induction: 10% Topical induction: 50% Challenge: 50%
Vehicle	Aqua ad iniectabilia
Pretreatment prior to topical application	Yes (sodium lauryl sulfate) / No
Reliability check	Benzocaine (2% intradermal induction, 5% topical induction and 5% challenge)
Remarks	None

Results and discussions

Table B.6.2-3: Skin sensitisation of Virgo

	24 hours	**48 hours**
	After challenge*	
Virgo	0/10	0/10
Test Vehicle Control Group	0/10	0/10
Positive control	20/20	20/20

* Number of animals with positive dermal response (scores of 1-3)/number of animals in dose group

Clinical signs:	None

Conclusion

Under the experimental conditions, Virgo is not a skin sensitiser. Thus, no classification is required according to Regulation (EC) No. 1272/2008.

Conclusion by the RMS (2019):

The study is still considered to be acceptable although the undiluted product was not tested. No skin

reactions were observed after challenge with 50% test item and it is not expected that a clear positive result would have been observed when applying the undiluted product for the topical induction and challenge. However, as this test is not considered to be appropriate for detecting a sensitising potential of micro-organisms including viruses the product needs to be labelled with the following phrase: "Contains *Cydia pomonella* Granulovirus. Micro-organisms may have the potential to provoke sensitizing reactions."
The notifier recommends not using this warning phrase since no positive results were yet published for CpGV or other viral species currently approved in the EU (Martel et al, 2010, ASB2011-9441; Hackl et al., 2015, ASB2015-4072). However, as already stated in the first evaluation of the study in the DAR from 2007, it cannot be ruled out that proteins from the larval matrix material in the technical active substance might provoke sensitisation. Therefore, the RMS is of the opinion to keep the warning phrase.

B.6.3 Data on exposure

Virgo is applied up to 6 times against codling moth on pome fruit and walnut at a maximum rate of 1.5×10^{13} GV/ha.

No toxicological reference value has been derived for *Cydia pomonella* Granulovirus since toxicity, pathogenicity or infectivity in mammals has not been observed for this virus (e.g.). CpGV is naturally present in the environment. Hence, a risk for operators, workers, bystanders and residents is not expected when the product is used as intended.

Regarding the potential risk for sensitisation PPE is required for the operator.

B.6.4 Available toxicological data relating to non-active substances

Toxicological information on the co-formulants is presented in Vol. 4. No additional classification is required.

B.6.5 Supplementary studies for combinations of plant protection products

Not necessary as no combinations of plant protection products are recommended.

B.6.6 Summary and evaluation of health effects

The toxicological studies on CpGV and the formulated product Carpovirusine reveal that no health risks have to be anticipated for operators, workers, bystanders and residents.

Due to a potential risk for sensitisation operators will have to wear PPE, which will reduce exposure.

B.6.7 References relied on

Data point	Author(s)	Year	Title Owner, Report No. Source (where different from owner) GLP or GEP status Published or not	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
KMP 7.1.1	[redacted]	2005	ACUTE ORAL TOXICITY STUDY OF VIRGO IN CD RATS Sipcam S.p.A., 18974/05 [redacted] GLP: yes Published: no BVL-3538436, TOX2006-1692	yes	no	not protected	SIP	Y KIIIM 7.1.1
KMP 7.1.2	[redacted]	2005	ACUTE INHALATION TOXICITY STUDY OF VIRGO IN RATS Sipcam S.p.A., 19016/05 [redacted] GLP: yes Published: no BVL-3538437, TOX2006-1054	yes	no	not protected	SIP	Y KIIIM 7.1.3
KMP 7.1.3	[redacted]	2005	ACUTE DERMAL TOXICITY STUDY OF VIRGO IN CD RATS Sipcam S.p.A., 18975/05 [redacted] GLP: yes Published: no BVL-3553325, TOX2006-1058	yes	no	not protected	SIP	Y KIIIM 7.1.2

Data point	**Author(s)**	**Year**	**Title** **Owner, Report No.** **Source (where different from owner)** **GLP or GEP status** **Published or not**	**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**
KMP 7.2.1	[redacted]	2005	ACUTE DERMAL IRRITATION/CORROSION TEST (PATCH TEST) OF VIRGO IN RABBITS Sipcam S.p.A., 18976/05 [redacted] GLP: yes Published: no BVL-3538438, TOX2006-1059	yes	no	not protected	SIP	Y KIIIM 7.1.4
KMP 7.2.2	[redacted]	2005	ACUTE EYE IRRITATION/CORROSION TEST OF VIRGO IN RABBITS Sipcam S.p.A., 18977/05 [redacted] GLP: yes Published: no BVL-3538439, TOX2006-1060	no	no	not protected	SIP	Y KIIIM 7.1.5
KMP 7.2.3	[redacted]	2005	EXAMINATION OF VIRGO IN THE SKIN SENSITISATION TEST IN GUINEA PIGS ACCORDING TO MAGNUSSON AND KLIGMAN (MAXIMISATION TEST) Sipcam S.p.A., 18978/05 [redacted] GLP: yes Published: no BVL-3538440, TOX2006-1050	yes	no	not protected	SIP	Y KIIIM 7.1.6

Data point	Author(s)	Year	Title Owner, Report No. Source (where different from owner) GLP or GEP status Published or not	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
KMP 7.2.3/02 1. additional submission	Hackl, E., Pacher-Zavisin, M., Sedman, L., Arthaber, S., Bernkopf, U., Brader, G., Gorfer, M., Mitter, B., Mitropoulou, A., Schmoll, M., van Hoesel, W., Wischnitzky, E., Sessitsch, A.	2015	LITERATURE SEARCH AND DATA COLLECTION ON RA FOR HUMAN HEALTH FOR MICROORGANISMS USED AS PLANT PROTECTION PRODUCTS REFERENCE not available, not stated EFSA Journal, 2015 EN-801, 173pp GLP/GEP: no Published: yes BVL-3306739, ASB2015-4072	no	no	not protected	-	N
KMP 7.2.3/03 1. additional submission	Martel, C., Nielsen, G.D., Mari, A., Licht, T.R., Poulsen, L.K.	2010	BIBLIOGRAPHIC REVIEW ON THE POTENTIAL OF MICROORGANISMS, MICROBIAL PRODUCTS AND ENZYMES TO INDUCE RESPIRATORY SENSITIZATION not available, not applicable EFSA Eur. Food Saf. Auth., CFP/EFSA/FEEDAP/2009, 1-95 GLP/GEP: no Published: yes BVL-3306740, ASB2011-9441	no	no	not protected	-	N
KMP 7.2.3/04 1. additional submission	Krieg, A.	1976	GRANULOSIS AND NUCLEAR POLYHEDROSIS VIRUSES: SAFETY ASPECTS CONCERNING THER PRODUCTION AN APPLICATION [GERMAN ORIGINAL] not available, not applicable Z. angew. Entomol., 82, 129-134 GLP/GEP: no Published: yes BVL-3306966, BVL-3146322, BWS2003-90	no	no	not protected	-	N