Renewal Assessment Report

Cydia pomonella GV

Volume 3 – B.2 Biological properties

Rev. 0 – 16 October 2020

Rapporteur Member State: Germany

Co-Rapporteur Member State: The Netherlands

Version history

When	What
16 October 2020	First version submitted to EFSA

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

Table of contents

B Summary of the data and information

B.2 Biological properties of the micro-organism 6

B.2.1 History of the micro-organism and its uses. Natural occurrence and geographical distribution 6
B.2.1.1 Historical background 6
B.2.1.1.1 Conclusion by the RMS (2019): 7
B.2.1.1.2 Cited references 8
B.2.1.2 Origin and natural occurrence 11
B.2.1.2.1 Conclusion by the RMS (2019) 12
B.2.1.2.2 Cited references 13
B.2.2 Information on target organisms 16
B.2.2.1 Description of target organisms 16
B.2.2.1.1 Conclusion by the RMS (2019) 17
B.2.2.1.2 Cited references 18
B.2.2.2 Mode of action 18
B.2.2.2.1 Conclusion by the RMS (2019) 21
B.2.2.2.2 Cited references 23
B.2.3 Host specificity range and effects on species other than the target harmful organism 26
B.2.3.1 Conclusion by the RMS (2019) 28
B.2.3.2 Cited references 29
B.2.4 Development stages/life cycle of the micro-organism 32
B.2.4.1 Conclusion by the RMS (2019) 33
B.2.4.2 Cited references 36
B.2.5 Infectiveness, dispersal and colonisation ability 39
B.2.5.1 Effects of sunlight 39
B.2.5.2 Effects of temperature 42
B.2.5.3 Effects of humidity 43
B.2.5.4 Effects of substrate: 44
B.2.5.5 Dispersal routes 45
B.2.5.6 Conclusion by the RMS (2019) 45
B.2.5.7 Cited references 47
B.2.6 Relationships to known plant or animal or human pathogens 51
B.2.6.1 Conclusion by RMS 52
B.2.6.2 Cited references 52
B.2.7 Genetic stability and factors affecting it 53
B.2.7.1 Conclusion by the RMS (2019) 55
B.2.7.2 Cited references 56
B.2.8 Information on the production of metabolites (especially toxins) 64
B.2.8.1 Conclusion by the RMS (2019) 64
B.2.8.2 Cited references 65
B.2.9 Antibiotics and other anti-microbial agents 65
B.2.10 References relied on 67
B.2.10.1 Summary of literature search performed by the notifier 67
B.2.10.2 Evaluation of literature search by the RMS (2019) 70
B.2.10.3 References 76

INTRODUCTION

The companies Andermatt Biocontrol GmbH, Arysta Life Science S.A.S., and Serbios srl have agreed on the formation of a Task Force in order to submit a dossier for the renewal of approval of the micro-organism *Cydia pomonella* Granulovirus (CpGV) as an active substance in compliance with Regulation (EU) No 844/2012 and Regulation (EC) No 1107/2009.

The initial dossiers for inclusion of *Cydia pomonella* Granulovirus into Annex I of Commission Directive 91/414 were submitted to the authorities of Germany as rapporteur member state in November 2005. Andermatt Biocontrol GmbH and Probis GmbH together as a Task Force, Arysta LifeSience S.A.S. and Sipcam S.p.A. were the notifiers in the initial evaluation of approval of CpGV as active substance. Serbios srl has acquired all data and registrations concerning CpGV and formulated products from Sipcam S.p.A..

Inclusion of the first isolate of *Cydia pomonella* Granulovirus (Mexican isolate) into Annex I (now list of approved active substances) entered into force on 01 May 2009 (Commission Directive 2008/113/EC[1]). This active substance is an approved active substance under Regulation (EC) 1107/2009 (repealing Commission Directive 91/414/EEC) as specified in Commission Implementing Regulation (EU) 540/2011 of 25 May 2011 and Commission Implementation Regulation (EU) No 880/2014 amending Commission Implementation Regulation (EU) No 540/2011. Further isolates were added to Annex I following evaluation according to the "Guidance Document SANCO/0253/2008 rev. 2 on the assessment of new isolates of baculovirus species already included in Annex I of Council Directive 91/414" in May 2011, when the Standing Committee on the Food Chain and Animal Health (SCFCAH) took note of the amended review report of 5 May 2011.

This dossier comprises the following isolates: the Mexican isolate CpGV-M, CpGV-V01, CpGV-V03, CpGV-V14, CpGV-V15, CpGV-V22, CpGV-V45, and CpGV-R5.

Cydia pomonella Granulovirus (CpGV) belongs to the group of baculoviruses. The inclusion of data from other baculoviruses in this dossier is justifiable due to this group relationship. Baculoviruses and CpGV in particular have been used for decades as plant protection products to control diverse pest insects. CpGV acts highly specific against larvae of the codling moth *Cydia pomonella* and some isolates such as CpGV-V22, CpGV-V45, and CpGV-R5 can infest the Oriental fruit moth *Grapholita molesta* or the plum fruit moth *Grapholita funebrana*. The mode of action of CpGV is a bi-phasic infection process of the larval stages of the above cited hosts. After oral ingestion of viral occlusion bodies, the virus replicates in the midgut cells (primary infection) and then infection is spread via non-occluded viruses to other body tissues (secondary infection) leading to the insect's death. CpGV is not supposed to have any harmful effects on organisms not belonging to the family of Tortricidae[2]. With regard to environmental safety it is important to note that CpGV and the whole group of baculoviruses are naturally present in the environment. The experience that baculoviruses present no risk to mammals and men has been confirmed by numerous studies. The family of baculoviruses is regarded to be safe for humans and vertebrates confirmed by the inclusion of this virus family in the list of "Qualified Presumption of Safety" published by EFSA[3]. Therefore, their application in pest control means only a fluctuation of the virus titre in the biotope of the pest insect. CpGV and the whole family of baculoviruses are not related to any animal (other than arthropods) or plant pathogen and it does not produce any metabolite[2]. For these reasons, no harmful effects from CpGV on humans, other vertebrates, other non-target organisms or the environment are expected. According to Commission Regulation (EU) 2016/439[4] *Cydia pomonella* Granulovirus is included into Annex IV of Regulation (EC) No 396/2005[5]. This means that no residue definition applies to the microorganism and no MRL is set for any of the existing or intended uses.

[1] OJL 330, 09.12.2007, p.6
[2] OECD, 2002. Consensus document on information used in the assessment of environmental applications involving baculoviruses. ENV/JM/MONO(2002)1 33, 1–16. doi:ENV/JM/MONO(2007)10
[3] EFSA Journal 2015; 13(12):4331
[4] OJL 78, 23.03.2016, p. 31-33
[5] OJL 70, 23.02.2005, p.1-16

Data previously submitted for the Draft Assessment Report (DAR) by the notifier were previously evaluated by RMS Germany and presented in the Draft Assessment Report (DAR) (December 2007). For the Renewal Assessment Report (RAR) the notifier submitted new data and information based on recent literature searches and new studies. Information derived from the original DAR is highlighted in grey and the respective EU Point is complemented. Parts copied from the DAR are presented without any changes in this document.

Comment by the RMS (2019):
In the introductory part above the notifier states that *"CpGV is not supposed to have any harmful effects on organisms not belonging to the family of Tortricidae. With regard to environmental safety it is important to note that CpGV and the whole group of baculoviruses are naturally present in the environment. The experience that baculoviruses present no risk to mammals and men has been confirmed by numerous studies. The family of baculoviruses is regarded to be safe for humans and vertebrates confirmed by the inclusion of this virus family in the list of "Qualified Presumption of Safety" published by EFSA. Therefore, their application in pest control means only a fluctuation of the virus titre in the biotope of the pest insect. CpGV and the whole family of baculoviruses are not related to any animal (other than arthropods) or plant pathogen and it does not produce any metabolite. For these reasons, no harmful effects from CpGV on humans, other vertebrates, other non-target organisms or the environment are expected."*

This statement addresses several data requirements of Section B.2 which are, however, not separately dealt with later in this section. Therefore, the notifier should elaborate the necessary parts of this statement in more detail, particularly in chapters B.2.3, B.2.4 and B.2.8 and provide convincing references. It must also be noted that the notifiers' claim that CpGV does not produce any metabolites has not been backed up by any recent references. Moreover, neither "metabolite" nor "toxin" were included as specific search terms in the search strategy. However, a literature search covering the last ten years would be necessary to maintain the previous evidence that no metabolites and toxins are produced by neither CpGV nor *C. pomonella* upon infection with CpGV. Please refer also to the "References relied on" section (chapter B.2.10) for a summary and an evaluation of the literature search.

Nonetheless, RMS would like to point out that since the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA until September 2015, to which the notifiers refers in the above statement, no adverse information was published that would change the current "Qualified Presumption of Safety" status of any member of the Baculoviridae family (EFSA BIOHAZ Panel, 2018).

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Ricci, A., Allende, A., Bolton, D., Chemaly, M., Davies, R., Fernández Escámez, P. S., Girones, R., Koutsoumanis, K., Lindqvist, R., Nørrung, B., Robertson, L., Ru, G., Sanaa, M., Simmons, M., Skandamis, P., Snary, E., Speybroeck, N., Ter Kuile, B., Threlfall, J., Wahlström, H., Cocconcelli, P. S., Peixe, L., Maradona, M. P., Querol, A., Suarez, J. E., Sundh, I., Vlak, J., Barizzone, F., Correia, S., and Herman, L. (2018). Statement on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 8: suitability of taxonomic units notified to EFSA until March 2018. EFSA Journal 16 (7): 5315, 42 pp. https://doi.org/10.2903/j.efsa.2018.5315

B.2 Biological properties of the micro-organism

B.2.1 History of the micro-organism and its uses. Natural occurrence and geographical distribution

B.2.1.1 Historical background

The following information, highlighted in grey, is derived from the Draft Assessment Report Volume 3, Annex B-2, point B.2.1.1.1.

Natural occurring baculoviruses seem especially promising as they combine reasonable efficacy to very few hosts with environmental safety and no hazards to man, domestic animals, and wildlife.

The first attempts to use baculoviruses for biological control can be dated back to the year 1892. During massive population increases of nun moths (*Lymantria monocha*, L.), a severe pine pest in Europe, the use of the infectious agent was intended to combat the insect pest (OECD, 2002, BVL no 3682775).

Baculoviruses exclusively have been isolated from arthropods, primarily from 4 insect orders: Lepidoptera, Hymenoptera, Diptera, and Coleoptera (OECD, 2002). Any intended use of baculoviruses for insect pest management includes the screening for a virus isolate virulent for the particular species. Isolates from diseased insects in the application area frequently are the first choice. If available, such isolates are in general included in the screening, but the testing for suitable viruses is not conventionally limited to these indigenous agents (OECD, 2002 and references therein).

The CpGV-M isolate which is registered and commonly used for the control of the codling moth in different European countries and in the US was originally isolated in 1963 in nature from diseased insects on apple and pear trees found in Mexico (near Valle de Allende, Chihuahua; OECD, 2002 and references therein). The virus identified by Tanada (CpGV) was then described and multiplied in Berkeley.

The first tests in California using CpGV-M proved its high virulence against *Cydia pomonella* larvae. Field trials were set up during the seventies in Canada, in Switzerland, in Germany, and in Australia. In 1980, Sandoz Inc. started up an experimental production of CpGV and obtained an authorisation for experimentation. However, development was stopped in 1982 (Huber, 1990, BVL no 2019086).

In France, the INRA (National Institute for Agronomical Research), within the framework of the search of alternative control methods, has developed the standard product, the CARPOVIRUSINE. Arysta LifeScience S.A.S., formerly CALLIOPE S.A.S., together with INRA, has undertaken the registration and development of CARPOVIRUSINE (Huber, 1990).

In Switzerland, Andermatt-Biocontrol developed a CpGV formulation named MADEX which received the registration in 1987. Hoechst developed its own CpGV product in Germany under the name of Granupom in 1989 (Huber, 1990).

In 1984, Naser *et al.* cultured CpGV in cell lines of the codling moth (Tanada *et al.*, 1993 and references therein, BVL no 3682776). The virus is produced by infecting codling moth larvae, harvesting the infected organisms and extracting the granular occlusion bodies by centrifugation. This technique requires very large quantities of larvae for virus production and is, thus, an expensive procedure. Trials are underway on the production of the virus in a more cost-effective way (Copping, 1998, BVL no 3682777).

A single CpGV-infected insect can often contain a mixture of virus genotypes. Isolation of single genotypes is possible through in vitro plaque assay (Jones, 2000, BVL no 3682778). However this is not possible for viruses for which there is no established cell culture system. Moreover, in vitro culture

requires expensive facilities. A simple but effective alternative is to use the in vivo dilution technique. With this technique a low dose of a virus isolate is given to early instar insects. Virus is collected individually from the insects that die and the process repeated preferably at least three times. At each stage the REN (restriction endonuclease) profile of the virus collected from each insect is obtained. The concept is that at a low dose, a certain percentage of young larvae will be infected by a single virus particle, which will lead to the insect dying from a single CpGV genotype.

A detailed continent-by-continent survey on the developmental, experimental and commercial use of baculovirus insecticides was compiled by Hunter-Fujita *et al.* (OECD, 2002 and references therein). Some examples of the most important baculovirus insecticides tested and used in the field are: *Adoxophyes orana* GV, *Agrotis segetum* GV, *Anticarsia gemmatilis* MNPV, *Autographa californica* MNPV, *Heliothis* (*Helicoverpa*) sp. NPV, *Helicoverpa armigera* NPV, *Lymantria dispar* NPV, *Mamestra brassicae* NPV, *Neodiprion sertifer* NPV, *Orgyia psuedotsugata* NPV and *Spodoptera* spp. NPV.

New data 2016

No new data have been submitted under this point. The notifier considers the previously submitted information to be acceptable to cover current requirements. No additional references were identified by the notifier from peer-reviewed open literature to be relevant for the historical background of *Cydia pomonella* Granulovirus. Please refer to the "References relied on" section (chapter B.2.10) for a summary and an evaluation of the literature search.

B.2.1.1.1 Conclusion by the RMS (2019):

According to the data requirements laid down by Commission Regulation (EU) No 283/2013 (Annex Part B Micro-organisms including viruses) the following point applies for chapter B.2.1.1:

> *"The historical background of the micro-organism and its use (tests/research projects or commercial use) must be provided"*
>
> While RMS considers this data requirement in general as fulfilled for peer-reviewed open literature published until 2002, RMS doubts that no new information has been become available ever since.

The notifier's conclusion that no additional references were identified from peer-reviewed open literature to be relevant for the chapter on the historical background of the micro-organism is evident as the literature search strategy did neither include specific search terms like "history" OR "historical" OR "histor?" nor "commercial AND use" or other search terms that could in any way be relevant for the historical background of the virus. It is highly unlikely that nothing has been published in the peer-reviewed open literature on the historical background of something as successful as the CpGV since publication of the OECD Consensus Document in 2002. For example a recent and comprehensive review on CpGV by Lacey *et al.* (2008) covers not only the biology but also the use of CpGV as a biocontrol agent (Lacey, L. A., Thomson, D., Vincent, C., and Arthurs, S. P. (2008): Codling moth granulovirus: a comprehensive review, Biocontrol Science and Technology: 18, 639-663, https://doi.org/10.1080/09583150802267046).

Moreover, the notifier cites the first edition of "The BioPesticide Manual" (Copping, 1998) declaring that "trials are underway on the production of the virus in a more cost-effective way". This manual has meanwhile several times been revised. The most recent edition (fifth edition) has been published in 2014 under the title "Manual of Biocontrol Agents" and an online version containing up-to-date information has also been made available by the publisher. It can be assumed that 20 years after its first publication a cost-effective production of the virus has been established; thus, the statement that "trials are underway" may have been relevant for the DAR but is meanwhile clearly obsolete. Finally, RMS wonders

whether the "in vivo dilution technique" described by Jones (2000) to isolate a single virus from a mixture of virus isolates has been relevant for the isolation of CpGV from codling moths or still is for the isolation of new strains. If so this should clearly be stated.

It should also be noted that "baculoviruses" infecting Coleoptera which were still considered as baculoviruses by the OECD Consensus Document (OECD, 2002, BVL no 3682775) do not belong any longer to the baculoviruses according to current virus taxonomy (International Committee on Taxonomy of Viruses – ICTV) but are classified as Nudiviruses (family Nudiviridae); https://talk.ictvonline.org/taxonomy. According to Herniou *et al.* (2011), baculoviruses only infect members of Lepidoptera, Hymenoptera and Diptera (Herniou, E. A., Arif, B. M., Becnel, J. J., Blissard, G. W., Bonning, B., Harrison, R., Jehle, J. A., Theilmann, D. A. and Vlak, J. M., Baculoviridae. In: King, A. M. Q., Adams, M. J., Carstens, E. B., Lefkowitz, E. J. (editors). *Virus Taxonomy*. Oxford: Elsevier; 2011. pp. 163–174.

B.2.1.1.2 Cited references

Report KMA 2.1.1 – OECD (2002), Consensus Document on Information used in the Assessment of Environmental Applications involving *Baculovirus* (ENV/JM/MONO(2002)1), Series on Harmonization of Regulatory Oversight in Biotechnology, No.20, OECD Environment Directorate, Paris. (Available on the Biotrack website at http://www.oecd.org/biotrack/)
Published report
BVL no 3682775

Abstract: The OECD's Working Group on Harmonization of Regulatory Oversight in Biotechnology decided at its first session, in June 1995, to focus its work on the development of consensus documents which are mutually acceptable among Member countries. These consensus documents contain information for use during the regulatory assessment of a particular product. This document contains general information on baculoviruses such as organism characteristics, behavior in the environment, their history of use and interactions, as well as environmental safety considerations. Germany served as lead country in the preparation of this document. It has been revised on a number of occasions based on the input from other Member countries. It is intended for use by regulatory authorities and others who have responsibility for assessments and by those who are actively involved with genetic improvement and intensive management of the genus.

Previous evaluation: **in DAR (2007)**
Evaluation by the RMS (2019): **Relevant and reliable.**

Report KMA 2.1.1 – Huber, J. (1990), History of the CpGV as a Biological Control Agent - Its Long Way to a Commercial Viral Pesticide, In: Vth International Colloquium on Invertebrate Pathology and Microbial Control, incorporating the XXIIIrd Annual Meeting of the Society for Invertebrate Pathology, Proceedings and Abstracts, Adelaide, Australia, 20-24 August 1990. pp.424-427
Published report
BVL no 2019086

Abstract: The story of the granulosis virus (CpGV) of the codling moth, *Cydia pomonella*, (CpGV) the first and only insect virus to become fully registered for use on food crops, reads like the pedigree of a plant or animal species, with many side branches, some of them dying or staying dormant for some time. Everything started in September 1963, when L.E. CALTAGIRONE from the University of California in Berkeley, on his way back from a trip to northern Mexico, where he had collected parasitized codling moth larvae, stopped for a brake at the road side outside Valle de Allende. There were some abandoned apple and pear trees near by and CALTAGIRONE used his time to collect there a few diapausing larvae of codling moth. Back in Berkeley, the larvae were maintained in the laboratory and observed for the development of parasites or diseases. Two individuals from the larvae collected near Valle de Allende died from a virus which subsequently was identified by TANANA (1964) to be a new granulosis virus. This was the beginning of a long history of research on the virus, involving

scientists from all the continents, which finally led, more than twenty years later, to the registration of the CpGV as a viral pesticide for control of codling moth.

Previous evaluation: **in DAR (2007)**
Evaluation by the RMS (2019): **Relevant and reliable**

Report KMA 2.1.1 – Tanada, Y., Kaya, H.K. (1993), DNA-Viral Infections: Baculoviridae, In: Vega, F. and Kaya, H.K. (ed.), Insect Pathology, pp. 171-244. London: Academic Press Inc.
Published report
BVL no 3682776

Abstract: The word *virus* is derived from Latin and means a slimy liquid, poison, or stench.The early definition of a virus was based on submicroscopic size and obligate pathogenicity. More recently, the definitions attempted to convey two qualities of the virus: (1) possession of its own genetic material, which inside the host cell behaved as part of the cell, and (2) presence of a submicroscopic infective stage, the virion, which served as the vehicle for introducing the viral genome into a cell (Lwoff and Tournier 1971). These definitions, however, did not adequately separate viruses from other minute parasitic procaryotes, such as rickettsiae, mycoplasma, and chlamydia. Matthews (1991) has thoroughly discussed the characterization of these procaryotes and their differences from the virus. He defined a virus as follows:

A virus is a set of one or more nucleic acid template molecules, normally encased in a protective coat or coats of protein or lipoprotein, that is able to organize its own replication only within suitable host cells. Within such cells, virus replication is (i) dependent on the host's protein-synthesizing machinery, (ii) organized from pools of the required materials rather than by binary fission, (iii) located at sites that are not separated from the host cell contents by a lipoprotein bilayer membrane, and (iv) continually giving rise to variants through various kinds of change in the viral nucleic acid.

The virus must normally be transmissible and cause disease in a host. Viral diseases are one of the most widely investigated infections in insects. These studies have resulted because of the extensive basic and applied interests in viruses and from the development of elaborate and complex equipment, including the sophisticated techniques in biochemistry, serology, pathology, tissue culture, and recombinant DNA technology. With these advances, applied insect virology has extended beyond pest control into the field of genetic engineering, where the virus serves a vector for the expression of foreign genes to produce biochemically and pharmaceutically important products. The accomplishments in genetic engineering are due primarily to the availability of invertebrate cell lines. Up to 1989, cell lines have been formed from 55 species of invertebrates in seven orders within Arthropoda and with most of them from Lepidopter and Diptera (Hink and Hall 1989).

Previous evaluation: **in DAR (2007)**
Evaluation by the RMS (2019): **Relevant and reliable, but possibly not reflecting the current state of knowledge. A revised edition (2nd edition) of this book was published in 2012. Chapter 4 of the 2nd edition, i.e. "Baculoviruses and Other Occluded Insect Viruses"by Harrison⁎and Hoover would perhaps be the more appropriate source (see links provided below)**

https://www.elsevier.com/books/insect-pathology/vega/978-0-12-384984-7
https://doi.org/10.1016/B978-0-12-384984-7.00004-X

Report KMA 2.1.1 – Copping, L.G., (1998). 1:36 *Cydia pomonella* granulosis virus. In: Copping, L.G. (ed.) (1998), The BioPesticide Manual. A world compendium, 1st edition, British Crop Protecion Council, pp. 60-61, Farnham, UK
Published report
BVL no 3682777

Abstract: The nomenclature of *Cydia pomonella* granulosis virus, its source, production, target pests and target crops, its biological activity, commercialization, application, product specifications, and compatibility, as well as its mammalian toxicity, environmental impact and non-target toxicity are shortly summarized.

Previous evaluation:	**in DAR (2007)**
Evaluation by the RMS (2019):	**Relevant for the DAR but possibly not reflecting the current state of knowledge. This manual has several times been revised. The most recent edition (fifth edition) has been published in 2014. Furthermore, an online version containing up-to-date information has been made available by the publisher (see links provided below).** https://www.bcpc.org/product/manual-of-biocontrol-agents-fifth-edition https://www.bcpc.org/product/manual-of-biocontrol-agents-online

Report KMA 2.1.1 – Jones, K.A. (2000), Bioassays of Entomopathogenic Viruses, In: Navon, A., Ascher, K.R.S. (eds.), Bioassays of entomopathogenic microbes and nematodes, pp. 95-140, CABI Publishing
http://dx.doi.org/10.1079/9780851994222.0095
Published report
BVL no 3682778

Abstract: This chapter outlines the many different bioassay techniques that are used with insect viruses, such as *Spodoptera littoralis* nuclear polyhedrosis viruses. The need to find out an accurate estimate of viral infectivity requires a more labour-intensive approach than the need to compare the activity of different viral formulations. However, all the techniques can give statistically valid results if applied correctly. There are many variations on the described methodologies. Further variations will be developed for new insect-virus systems. Future techniques will also involve adaptations of bioassay techniques used for other stomach poisons or microbes.

Previous evaluation:	**in DAR (2007)**
Evaluation by the RMS (2019):	**Relevant and reliable but possibly not reflecting the current state of knowledge. Notably, the copy provided is hardly legible.**

Report KMA 2.1.1 – Shao-Hua, C., Hong-Liang, S., Zuo-Hu, L. (1998), Effect of Temperature Oscillation on Insect Cell Growth and Baculovirus Replication, Applied and Environmental Microbiology, 64, 2237-2239
Published report
BVL no 3682779

Abstract: Temperature oscillation can enhance cell viability of sf9 insect cells and baculovirus production of occlusion bodies (OB) and extracellular virus (ECV) compared with constant temperature in stationary culture and suspension culture. The optimal oscillation range was 24 to 28°C. At this temperature oscillation, the viability of uninfected and infected sf9 cells can be maintained much longer than at 28°C. Although the rate of virus infection was a little low at 24 to 28°C, the final cell infectivity was similar to that at a constant temperature of 28°C. The production of OB was increased from 13.4 to 17.4/cell in stationary culture and from 13.9/cell to 18.1/cell in suspension culture. The

titer of ECV was increased from 87 to 114 PFU/cell in stationary culture and from 79 to 114 PFU/cell in suspension culture.
Previous evaluation: in DAR (2007) **Evaluation by the RMS (2019): Not relevant as CpGV (and other baculovirus active substances) are all produced *in vivo* and not in cell culture**

B.2.1.2 Origin and natural occurrence

The following information, highlighted in grey, is derived from the Draft Assessment Report Volume 3, Annex B-2, point B.2.1.1.2.

The CpGV isolate in use derives from Mexico and was originally isolated from diseased insects on apple and pear trees found in Mexico (near Valle de Allende, Chihuahua; OECD, 2002 and references therein, BVL no 2019088). Baculoviruses are ubiquitous in the environment, their prevalence depending on the frequency of occurrence of their arthropod hosts that inhabit terrestrial and marine ecosystems (OECD, 2002). Their geographic distribution usually corresponds to the distribution of their hosts. Their application in pest control means only a fluctuation of the virus titre in the biotope of the pest insect (Krieg, 1976, BVL no 3682706).

New data 2016/2017:

Like all Baculovirus isolates used in biological control, the Mexican isolate CpGV-M is genetically heterogeneous. It consists of a mixture of similar genotypes, which differ in the presence or absence of insertions or deletions or by point mutations. Genetically homogenous strains are very difficult to obtain and even not desired to account for variations in host susceptibility. Companies have selected new isolates for individual properties. These isolates were added to Annex I following evaluation according to the "Guidance Document SANCO/0253/2008 rev. 2 on the assessment of new isolates of baculovirus species already included in Annex I of Council Directive 91/414" in May 2011, when the SCFCAH took note of the amended review report of 5 May 2011.

The new isolate CpGV-V01 (CpGV-Madex Plus) was selected from the CpGV-M isolate used in MADEX (Kessler, 2008a, BVL no 3306439; Jehle, 2006, BVL no 3306435; for a summary and an evaluation of the study by Jehle (2006) please refer to Volume 3 → B1 "Identity of the micro-organism" → B.1.3 "Name and species description, strain characterization"). The CpGV isolate CpGV-V01 was obtained without genetic modifications. CpGV-V01 shows high efficacy against *C. pomonella* populations who are resistant against CpGV-M, comparable to the efficacy of the original MADEX against susceptible *C. pomonella*. The efficacy of CpGV-V01 and CpGV-M against susceptible populations is similar (Please refer to Volume 4 of Andermatt Biocontrol GmbH → C.1 "Confidential information").

The new isolate CpGV-V03 has been "conventionally" selected and does not contain genetic modifications. For further information on the isolate, please refer to Volume 4 of of Andermatt Biocontrol GmBH → C.1 "Confidential information" → C.1.2 "Detailed specification of the micro-organism". The isolate does not have any characteristics differing from the typical description of the species and differs from CpGV-M only in the ability to break the resistance of *C. pomonella* populations that are resistant to CpGV-M.

As other CpGV isolates, CpGV-V14 is infective to *C. pomonella* and to some extend to *Cryptophlebia leucotreta*, but is in contrast to other isolates not infective to the closely related tortricid species *Grapholita molesta.* The isolate does not have any phenotypic characteristics differing from the typical description of the species. It differs from CpGV-M in the ability to break the resistance of *C. pomonella* populations that are resistant to CpGV-M and other CpGV-M like isolates. The new isolate CpGV-V14 does not contain genetic modifications.

The isolate CpGV-V15 has been isolated from *C. pomonella* larvae using classical selection methods (Kessler, 2010a, BVL no 3306437). The isolate does not have any characteristics differing from the typical description of the species and differs from CpGV-M only in the ability to break the resistance of *C. pomonella* populations that are resistant to CpGV-M (Jehle and Eberle, 2009a, BVL no 3306433; for a summary and an evaluation of the study please refer to Volume 3 → B1 "Identity of the micro-organism" → B.1.3 "Name and species description, strain characterization").

The new isolate CpGV-V22 was obtained from infested *C. pomonella* larvae using classical selection methods and does not contain genetic modifications (Kessler, 2010b, BVL no 3306438). Genetically, CpGV-V22 is closely related to CpGV-M and belongs to the same genome type A as CpGV-M (Jehle and Eberle, 2009b, BVL no 3306434; for a summary and an evaluation of the study please refer to Volume 3 → B1 "Identity of the micro-organism" → B.1.3 "Name and species description, strain characterization"). In contrast to CpGV-M and other CpGV isolates, CpGV-V22 is infective to larvae of the oriental fruit moth, *Grapholita molesta* (Tortricidae). Like other CpGV isolates it is not infective to other tortricid species like *Adoxophyes orana*. The isolate does not have any other characteristics differing from the typical description of the species and the representative isolate CpGV-M.

As other CpGV isolates, the new isolate CpGV-V45 is infective to *C. pomonella* and *Grapholita molesta.* The isolate does not have any phenotypic characteristics differing from the typical description of the species. It differs from CpGV-M in the ability to break the resistance of *C. pomonella* populations that are resistant to CpGV-M and other CpGV-M like isolates. The new isolate CpGV-V45 does not contain genetic modifications.

The isolate CpGV-R5 was obtained by selection on *C. pomonella* larvae that are highly resistant against the Mexican isolate CpGV-M. CpGV-R5 is able to overcome resistance in *C. pomonella* populations that are resistant to CpGV-M. For further details on the selection of this isolate, please refer to Volume 4 of Arysta LifeScience S.A.S → C.1 "Confidential information".

From peer-reviewed open literature no additional references were identified by the notifier to be relevant for the origin and natural occurrence of *Cydia pomonella* Granulovirus. Please refer to the "References relied on" section (chapter B.2.10) for a summary and an evaluation of the literature search.

B.2.1.2.1 Conclusion by the RMS (2019)

According to the data requirements laid down by Commission Regulation (EU) No 283/2013 (Annex Part B Micro-organisms including viruses) four points need to be addressed in chapter B.2.1.2. These data requirements are outlined below followed by a short evaluation whether or not they have been adequately addressed by the notifier.

(i) "The geographical region and the place in the ecosystem (e.g. host plant, host animal, or soil from which the micro-organism was isolated) must be stated"

Addressed.

(ii) "The natural occurrence of the micro-organism in the relevant environment shall be given if possible at strain level"

While the origin of the CpGV isolates to be registered has briefly been outlined the notifier missed to mention that natural geographic isolates of CpGV are known from different areas world wide, including England, Russia, Canada, and Iran. Most if not all of these isolates consist of mixtures of genotypes closely related to each other. Several scientific papers dealing with geographic CpGV isolates have been published within the last ten years, e.g.

- Rezapanah, M., Shojai-Estabragh, S., Huber, J. Jehle, J. A. (2008). Molecular and biological characterization of new isolates of *Cydia pomonella* granulovirus (CpGV) from Iran. *Journal of Pest Science* 81, 187-191. https://dx.doi.org/10.1007/s10340-008-0204-2

- Eberle, K. E., Sayed, S., Rezapanah, M., Shojai-Estabragh, S., Jehle, J. A. (2009). Diversity and evolution of *Cydia pomonella* granulovirus (CpGV). *Journal of General Virology* 90, 662-671. https://dx.doi.org/10.1099/vir.0.006999-0

- Berling, M., Blachere-Lopez, C., Soubabere, O., Lery, X., Bonhomme, A., Sauphanor, B., and Lopez-Ferber, M. (2009). *Cydia pomonella granulovirus* genotypes overcome virus resistance in the codling moth and improve virus efficiency by selection against resistant hosts. Appl. Environ. Microbiol. 75, 925-930. https://dx.doi.org/10.1128/AEM.01998-08

- Arneodo, J. D., De Anna, J., Salvador, R., Farinon, M., Quintana, G. and Sciocco-Cap, A. (2015), Prospection and molecular analysis of CpGV isolates infecting *Cydia pomonella* at different geographical locations in Argentina. Ann Appl Biol, 166, 67-74. https://dx.doi.org/10.1111/aab.12162

- Zheng-Wei Wu, Jiang-Bin Fan, Huan Yu, Dun Wang and Ya-Lin Zhang (2015) Ultraviolet protection of the *Cydia pomonella* granulovirus using zinc oxide and titanium dioxide, Biocontrol Science and Technology, 25, 97-107, https://dx.doi.org/10.1080/09583157.2014.951029

(iii) "The method of isolation of the micro-organism shall be reported".

Addressed.

(iv) "In the case of a mutant, or a genetically modified micro-organism, detailed information should be provided on its production and isolation and on the means by which it can be clearly distinguished from the parent wild strain."

As all described isolates, i.e. CpGV-M, CpGV-15, CpGV-22, CpGV-V03, CpGV-V01 and CpGV-R5 have not been genetically modified, point (iv) does not apply.

RMS considers the search strategy as sufficient to cover literature relevant in regard to origin and natural occurrence of the virus as the specific term "host" was included in the search strategy. Even though other specific terms like "ecosystem", "origin", "occurrence" or alike were not considered, RMS is of the opinion that these terms are broadly covered by the former one. Yet, RMS is wondering why the papers concerning the natural occurrence of the micro-organism (suggested above) have either not been found by the notifier or were not considered relevant.

B.2.1.2.2 Cited references

Report KMA 2.1.2 – OECD (2002), Consensus Document on Information used in the Assessment of Environmental Applications involving *Baculovirus* (ENV/JM/MONO(2002)1), Series on Harmonization of Regulatory Oversight in Biotechnology, No.20, OECD Environment Directorate, Paris. (Available on the Biotrack website at http://www.oecd.org/biotrack/)
Published report
BVL no 2019088

Abstract: The OECD's Working Group on Harmonization of Regulatory Oversight in Biotechnology decided at its first session, in June 1995, to focus its work on the development of consensus documents which are mutually acceptable among Member countries. These consensus documents contain information for use during the regulatory assessment of a particular product. This document con-

tains general information on baculoviruses such as organism characteristics, behavior in the environment, their history of use and interactions, as well as environmental safety considerations. Germany served as lead country in the preparation of this document. It has been revised on a number of occasions based on the input from other Member countries. It is intended for use by regulatory authorities and others who have responsibility for assessments and by those who are actively involved with genetic improvement and intensive management of the genus.

Previous evaluation: **in DAR (2007)**
Evaluation by the RMS (2019): **Relevant and reliable.**

Report KMA 2.1.2 – Krieg (1976), Granulosis and Nuclear polyhedrosis viruses: Safety aspects concerning their production and application, Zeitschrift für Angewandte Entomologie, 82, 129-134
Published report
https://dx.doi.org/10.1111/j.1439-0418.1976.tb03382.x
BVL no 3682706

Abstract: *Baculoviruses* being highly specific agents are not supposed to have any harmful effect on personnel in research or industrial mass production of viral insecticides. The same is true for field application of such viruses as well as for consumption of virus treated crop. Considering safety problems it is important to note that *Baculoviruses* are naturally present in our environment. Therefore, their application in pest control means only a fluctuation of the virus titer in the biotop of the pest insect. The experience that contact of *Baculoviruses* with man or animals does not mean any risk for their health has been confirmed by special tests. For registration purposes, safety-tests are carried out according to WHO standards. In addition, tests on the potential infectivity have been made on mammalian cell cultures as well as concerning mutagenicity, teratogenicity, and oncogenicity on animals, all with negative results. Additional tests showed the harmlessness of *Baculoviruses* to honeybees and entomophagous insects.

Previous evaluation: **in DAR (2007)**
Evaluation by the RMS (2019): **Supplementary information. Not necessarily relevant in the context of origin and natural occurrence of CpGV.**

Report KMA 2.1.2/01 – Kessler, P. (2010a), Declaration of Origin CpGV isolate ABC-V15 (DSMZ GV-00013)
Not published
BVL no 3306437

Summary: The CpGV isolate ABC-V15, which has been deposit under the strain accession number DSMZ GV-00013, has been isolated from codling moth larvae after classical selection methods, in the laboratories of Andermatt Biocontrol AG, Stahlermatten 6, 6146 Grossdietwil, Switzerland.

Submitted for the purpose of renewal
Evaluation by the RMS (2019): **Relevant**

Report KMA 2.1.2/02 – Kessler, P. (2010b), Declaration of Origin CpGV isolate ABC-V22 (DSMZ GV-00014)
Not published
BVL no 3306438

Summary: The CpGV isolate ABC-V22, which has been deposit under the strain accession number DSMZ GV-00014, has been isolated from codling moth larvae after classical selection methods, in the laboratories of Andermatt Biocontrol AG, Stahlermatten 6, 6146 Grossdietwil, Switzerland.

Submitted for the purpose of renewal
Evaluation by the RMS (2019): **Relevant**

Report KMA 2.1.2/03 – Kessler, P. (2008), Declaration on the origin and characterisation of the active ingredient in MADEX Plus
Not published
BVL no 3306439

Summary: MADEX Plus is a product to control the larvae of *Cydia pomonella*. The active ingredient of MADEX Plus is *Cydia pomonella* Granulovirus (CpGV) that has been selected from the genetic pool of the CpGV Mexican isolate (CpGV-M). We confirm that the new CpGV genotype has been "conventionally" selected on MADEX-resistant host insects and that the new active ingredient is not genetically modified. A comparative restriction analysis revealed only slight difference between the selected CpGV strain used in MADEX Plus and the Mexican strain CpGV-M (Jehle, 2006). Two of four analysed restriction profiles were absolutely identical. In two other profiles, small variations have been detected, which indicates a more frequent presence of the previously described genotypes CpGV-E and CpGV-R in MADEX-Plus-CpGV.

Submitted for the purpose of renewal

Evaluation by the RMS (2019): Relevant

KMA 2.1.2/04 – Jehle, J., Eberle, K. (2009a), Comparative Restriction Analysis of V15
Not published
BVL no 3306433

Summary: For the identification of baculovirus isolates DNA endonuclease restriction (REN) analysis is usually used. By digesting viral DNA by different RENs specific restriction patterns can be identified and small genotypic variations can be located in a restriction map. In this study, DNA of V15 (Test item) was isolated and purified and subjected to endonuclease restriction analysis using the endonucleases *Sal*I, *Bam*HI, *Eco*RI and *Eco*RV. The restriction fragments were separated in an agarose gel and the obtained restriction profiles were compared to the restriction profiles of CpGV-M (Mexican isolate, propagated in Neustadt) and to published profiles of CpGV-M. It was found that the test item (V15) was a CpGV isolate containing at least two genome types. One of these genome types showed similarity to the REN profile of CpGV-E2 (Crook *et al.*, 1985, Eberle *et al.*, 2009), the other genome type differed from CpGV-M and E2. The different genome types seemed to be present a similar level in the mixture.

Submitted for the purpose of renewal as KMA 1.3/04

Evaluation by the RMS (2019): please refer to evaluation in Volume 3 → B1 "Identity of the micro-organism" → B.1.3 "Name and species description, strain characterization" → Report: KMA 1.3/04

KMA 2.1.2/05 – Jehle, J., Eberle, K. (2009b), Comparative Restriction and Phylogenetic Analysis of V22
Not published
BVL no 3306434

Summary: For the identification of baculovirus isolates DNA endonuclease restriction (REN) analysis is usually used. By digesting viral DNA by different RENs specific restriction patterns can be identified and small genotypic variations can be located in a restriction map. In this study, DNA of V22 (Test item) was isolated and purified and subjected to endonuclease restriction analysis using the endonucleases *Sal*I, *Bam*HI, *Eco*RI and *Eco*RV. The restriction fragments were separated in an agarose gel and the obtained restriction profiles were compared to the restriction profiles of CpGV-M (Mexican isolate, propagated in Neustadt) and to published profiles of CpGV-M. It was found that the test item (V22) was a CpGV isolate with a predominant A type genome profile. Faint submolar bands could be observed in the REN profile obtained with *Eco*RI and *Eco*RV, suggesting there is another genome type present in Test Item V22 at low level. Phylogenetic analysis of baculoviruses can be based on the

partial gene sequences of *late expression factor 8* (*lef-8*) and *polyhedrin/granulin* (*polh/gran*). Partial amplification of the *lef-8* and *polh/gran* followed by sequencing revealed no single nucleotide polymorphisms (SNPs) between V22 and CpGV-M. Phylogenetic analysis based on these partial sequences grouped V22 to GpGV-M. The main genome present in Test Item V22 can be attributed to A type genomes.
Evaluation by the RMS (2019): **Submitted for the purpose of renewal as KMA 1.3/05 please refer to evaluation in Volume 3 → B1 "Identity of the micro-organism" → B.1.3 "Name and species description, strain characterization" → Report: KMA 1.3/05**

KMA 2.1.2/06 – Jehle, J. (2006), Comparative Restriction Analysis of CpGV (Neustadt Mexican isolate) with CpGV (Madex plus) Not published BVL no 3306435
Summary: For the identification of baculovirus isolates DNA endonuclease restriction (REN) analysis is usually used. By digesting viral DNA by different RENs specific restriction patterns can be identified and small genotypic variations can be located in a restriction map. In this study, viral DNAs of CpGV (Mexican strain, (M-type), Neustadt) (Reference Item) and CpGV (Madex Plus) (Test Item) were isolated and purified and subjected to endonuclease restriction analysis using the endonucleases *Sal*I, *Bam*HI, *Eco*RI and *Eco*RV. The restriction fragments were separated in an agarose gel and the obtained restriction profiles were compared to each other and to published profiles of CpGV-M. It was found that the restricton profiles of CpGV (MadexPlus) differed from CpGV (Mexican strain, (M-type), Neustadt) in two out of four restriction digests. The additional restriction fragments observed for CpGV (Madex Plus) correspond to restriction patterns suggest that Madex Plus contains variants of CpGV which resemble the E and R type.
Evaluation by the RMS (2019): **Submitted for the purpose of renewal as KMA 1.3/06 please refer to evaluation in Volume 3 → B1 "Identity of the micro-organism" → B.1.3 "Name and species description, strain characterization" → Report: KMA 1.3/06**

B.2.2 Information on target organisms

B.2.2.1 Description of target organisms

Target organism of the CpGV isolates is the codling moth *Cydia pomonella* (L.)

The following information, highlighted in grey, is derived from the Draft Assessment Report Volume 3, Annex B-2, Point B.2.1.2.1.

Common names:	Codling Moth Carpocapse des Pommes et des Poires
Scientific name:	*Cydia pomonella*, L.
Order:	Lepidoptera
Family:	Tortricidae, subfamily Olethreutinae,
Synonyms:	*Carpocapsa pomonella*, *Laspeyresia pomonella*, *Enarmonia pomonella*

The codling moth is one of the most important pests in apple orchards. The greyish moth with a wing spread of about 2 cm and a characteristic cross band of chocolate brown deposits lays 50-75 eggs on the leaves, twigs and fruits. The egg laying period extends from the end of spring to summer. After hatching

(one to three weeks later), the first larval instars walk on the fruit, test the fruit by shallow stings and look for a site to enter the fruit (through the side of the apple, the calyx or near the stalk). The lesions are therefore visible from the end of the spring until the beginning of autumn. A partial entry of the larvae causes stings which alter the fruit quality. After complete penetration in the fruit, the larva bores a tunnel to the core of the fruit, and after complete development, exits (3 to 5 weeks later). They leave the fruit and seek suitable places for hiding, such as underneath bits of loose bark and other protected places mainly on the tree and seldom in the debris on the ground. Here cocoons are spun and pupation follows. Depending on climate, one, two or even more generations each year (in warm regions) are possible (Little, 1963, BVL no 3682781). Hibernation takes place in the form of diapausing larvae.

The larvae of the codling moth injure and contaminate the fruits by eating: the wormy fruit is familiar to everyone. Fruits very often drop prematurely, the remaining ones are not marketable. Mainly apples are attacked but to a smaller extent also pears, walnuts and occasionally other fruits may be affected.

New information 2016/2017:

Another target organism of the Mexican isolate and the new isolate CpGV-R5 of Arysta LifeScience S.A.S, as well as of the new isolates CpGV-V22 and CpGV-V45 of Andermatt Biocontrol AG is the oriental fruit moth, *Grapholita molesta*:

Common names:	Oriental fruit moth Oriental peach moth
Scientific name:	*Grapholita molesta* (Busck)
Order:	Lepidoptera
Family:	Tortricidae, subfamily Olethreutinae,
Synonyms:	*Cydia molesta, Carpocapsa molesta, Laspeyresia molesta*

Grapholita molesta is one of the main insect pests in stone fruits. It is native to China, but was introduced to Japan and North America and is now also found throughout of Europe, Asia and South America and in Hawaii, Morocco, Mauritius, South Africa, Australia and New Zealand. Adult moths are about 1 cm in length of greyish colour. The appearance of their larvae is very similar to larvae of the codling moth, but smaller (10-14 mm). The insect hibernates inside tightly woven cocoons in protected places on the tree e.g. bark scars or in the trash near the base of trees. First moths appear in spring and females lay about 50 single grey-white eggs on newly emerged shoots. At temperatures above 20°C larvae hatch after three to five days, at temperatures below 20°C larvae need about 10 days. Larvae of the first generation feed on terminals where they complete their development. Larvae of subsequent generations feed on shoot terminals and green fruit, and as fruit matures it becomes the preferred site of attack by this pest. Depending on climate, up to five generations per year can appear.

Larvae damage developing shoots and fruit. While feeding on shoots causes typical shoot strikes or flagging, the most severe damage occurs when larvae feed on fruits, which loose their marketability. Peach trees are the main host plant for *G. molesta* but also pear, apple, quince, nashi, plum, almond, cherries and apricot are attacked.

From peer-reviewed open literature no additional references were identified by the notifier to be relevant for the description of the target organisms of *Cydia pomonella* Granulovirus. Please refer to the "References relied on" section (chapter B.2.10) for a summary and an evaluation of the literature search.

B.2.2.1.1 Conclusion by the RMS (2019)

According to the data requirements laid down by Commission Regulation (EU) No 283/2013 (Annex Part B Micro-organisms including viruses) the following point applies for chapter 2.2.1:

"Details of harmful organisms against which protection is afforded, must be provided".

This point has been addressed, however, RMS would like to point out that no references were provided for the details on the oriental fruit moth *Graphita molesta.*

RMS considers the search strategy as sufficient to cover literature relevant in regard to the description of the target organisms as the specific term "host" was included in the search strategy.

B.2.2.1.2 Cited references

Report KMA 2.2.1 – Little, V.A. (1963), Orders Trichoptera and Lepidoptera, Family *Tortricidae* (Tortricids), The Codling Moth, In: – Little, V.A., General and Applied Entomology, 2nd edition, pp. 324-326, Harper & Row, New York
Published report
BVL no 3682781

Abstract: **Family *Tortricidae* (Tortricids).** This is a large family of rather small moths. There are probably more than 1,000 North American species. The front wings are broad and truncate at the outer margins. The larvae feed on a wide variety of plants. Many species are leaf-rollers, others feed in the fruits, buds or stems. A number of important pests are found in this group. This family is considered a superfamily, *Tortricoidea*, and subdivided into several families by a number of authorities. **The Codling Moth** (*Carpocapsa pomonella*). The codling moth (Fig. 199) is the most serious insect pest of apples, causing most of the wormy fruit which is so familiar to everyone. The core of the fruit is eaten out by the pinkish-white caterpillars which have brown heads are about ¾ inch long when fully grown. Apples, pears, English walnuts, and occasionally other fruits are attacked. This insect is found wherever apples are grown.

Previous evaluation: in DAR (2007)
Evaluation by the RMS (2019): **Relevant and reliable.**

B.2.2.2 Mode of action

The following information, highlighted in grey, is derived from the Draft Assessment Report Volume 3, Annex B-2, Point B.2.1.2.2.

Application of CpGV should be timed at hatching of larvae so that first-instar larvae on the surface of the fruits come in contact with the virus before entering the fruit, as the larval stage of the insect life cycle is the most susceptible to infection with baculoviruses.

After oral intake by the codling moth larvae, the granules are dissolved in the midgut and free virions are released which invade the midgut cells by fusion with the microvilli (Copping, 1998, BVL no 3714761). Histopathology studies revealed that two to three days after infection pathologies had developed in the fat body, hypodermis, tracheal matrix and the Malphigian tubules. Both, free virus rods and capsules, were found in these organs (Tanada & Leutenegger, 1968, BVL no 3714762).

Prior to cytopathological changes, the first reaction of fat body cells of *C. pomonella* is a sharp increase in RNA synthesis (mostly ribosomal RNA) associated with protein synthesis and localised to the swelling nucleolus (Tanada and Kaya, 1993 and references therein, BVL no 3714763). Then the nucleolus and chromatin degenerate, followed by the concomitant decrease in RNA and DNA syntheses to normal and subnormal levels. With the appearance of the virogenic stroma, there is a second resurgence of RNA synthesis of relatively long duration and a tremendous increase of DNA synthesis with a pronounced maximum at 60 to 70 h after infection.

Infected larvae cease to feed, become motionless and flaccid, and die (Bilimoria, 1986, BVL no 3689461). Some of the larvae with late infection continue to grow but, after having reached the fifth

stage, do not manage to form pupae. They eventually turn white in colour and die. The body of the insect liquefies and the virus is released into the environment where it can infect other codling moth larvae. The incubation period is independent of the dose of virus consumed by the insect. The various larval stages of the codling moth show different susceptibility: first-instar larvae are more sensitive to infection, and the tolerance increases with age until reaching its maximum at the fourth stage.

The virulence of CpGV to *Cydia pomonella* can be characterised by three parameters, which are the lethal concentration (LC_{50}), the lethal dose (LD_{50}) and the lethal time (LT_{50}). Steineke (2004, BVL no 2019090) gives an overview about different published values of lethal doses (LD_{50}) of L1 and L5 larvae presented in Table B.2.2-1 and lethal concentration (LC_{50}) presented in Table B.2.2-2 .

The lethal time (LT_{50}) of larvae depends on concentration of virus in the diet and on the temperature during the insect development. The values determined are presented in the TablesTable B.2.2-3 and Table B.2.2-4 (Steineke, 2004 and references therein).

Table B.2.2-1: Published values of lethal dose (LD_{50}) of L1 and L5 larvae

L1 larvae		L5 larvae	
LD_{50} [OB/larvae]	**Reference***	**LD_{50} [OB/larvae]**	**Reference***
1.4	Winstanley (Horticulture Research International, Wellesbourne, U.K., personal communication)	**10**	Winstanley (Horticulture Research International, Wellesbourne, U.K., personal communication)
1.5	Crook *et al.* (1984)	**50**	Etzel & Falcon (1976)
3	Crook *et al.* (1985)	**92**	Camponovo & Benz (1984)
28	with surfactant, Keller (1973)		
46	without surfactant, Keller (1973)		

*cited by Steineke (2004); OB – occlusion body

Table B.2.2-2: Published values of lethal concentration (LC_{50})

LC_{50} [OB/mL medium]	**Slope**	**Reference***
2.56×10^3	2.16	E. Fritsch & K. Undorf-Spahn (Institute for Biological Control, Darmstadt, Germany, personal communication)
2.73×10^3	2.12	Fritsch (1989)
2.40×10^3	1.38	Crook *et al.* (1984)
2.60×10^3	1.21	Crook *et al.* (1985)

*cited by Steineke (2004); OB – occlusion body

Table B.2.2-3: Values of lethal time (LT_{50}) of larvae that were exposed the first 24 hours to different concentrations of CpGV, and incubated at 26°C for 11 days

Concentration [OB/mL medium]	**Mortality [%]**	**LT_{50} [h]**	**Standard deviation**
2.5×10^3	27	114.5	0.0717
5.0×10^3	34	109.0	0.0709
10.0×10^3	57	104.6	0.0605

20.0 x 10^3	85	105.9	0.0862

OB – occlusion body

Table B.2.2-4: Values of lethal time (LT_{50}) of larvae that were exposed to 5000 OB/mL of medium with regard to temperature

Temperature [°C]	Mortality [%]	LT_{50} [h]	95% confident interval	Slope
19	95	467	445-492	0.007
22	90	230	218-242	0.015
24	93	201	192-210	0.022
26	95	190	182-200	0.021
28	95	174	166-182	0.024
30	81	172	162-183	0.017
32	50	298	264-382	0.008

For a common synthetic product, 1% of the normal dose would be totally ineffective. For CpGV, however, the low slope of the dose-effect-curve must be considered (Kienzle *et al.*, 2003, BVL no 3682783). Since with 1/10 of the normal concentration also rather good effects in damage control can be achieved, it seems realistic, that 1/100 of this concentration (1% of the CpGV applied) could be enough to cause a considerable but slow larval mortality as observed in experiments conducted by Kienzle *et al.* (2003).

The CpGV Task Force recommends a dosage of 0.15 and 0.33 x 10^{13} granules/ha and m crown height (number of applications: max. 6) for the products MADEX and Granupom, respectively.

Sipcam S.p.A. recommends a dosage of 6-15 x 10^{12} granules/ha (number of applications: 2-3) for their product VIRGO.

Arysta LifeScience S.A.S. recommends a dosage of 1 x 10^{13} granules/ha (number of applications: 1-10) for their product CARPOVIRUSINE.

Vertical transmission of CpGV

Steineke (2004) conducted a study to determine the possibility of transmission of CpGV by vertical transmission, i.e. from one generation to the following. L5 larvae from generation F0 were infected at sublethal levels (50 OB/larva). From these larvae only 18% became pupae. In the control the pupation rate was slightly higher (25%). The surviving insects were analysed by PCR to identify the presence of CpGV. After cross mating, the mortality rate and the infection rate were analysed. This study did neither clearly demonstrate nor prove vertical transmission of CpGV.

Horizontal transmission of CpGV

A possible scenario of horizontal transmission of CpGV is the contact between two larvae on the apple's surface. Larvae that are infected shortly after hatching have not tunneled far into the apple and die relatively close to the surface, so that their cadavers could provide a source of virus for healthy neonates exploring the apple's surface.

Repeated applications of *Cydia pomonella* (CpGV) can effectively control the codling moth (CM) in apple orchards. However, it is still unknown whether horizontal transmission of the virus from infected to uninfected larvae contributes to the efficacy of the virus insecticide. Horizontal transmission of CpGV was assayed using detached apples. In experiments using artificially applied virus dots on the apple's

surface or infected CM larvae as virus inoculum, it was found that the likelihood of infection of healthy CM larvae relied mainly on the larval behaviour. The amount of virus inoculum, either applied artificially or produced by the infected larvae, impacted the infection rate only to a small degree (Steineke 2004). Steineke and Jehle (2004, BVL no 2019092) proposed that horizontal transmission under field conditions is considerably lower than in laboratory experiments. Thus, horizontal transmission may be an unimportant factor for the efficacy of CpGV as biological control agent in managed orchards, where high densities of codling moths are never reached. In unmanaged orchards, however, where high codling moth densities are more common, horizontal transmission could help maintain the disease within a season (Steineke and Jehle, 2004).

New data 2016

No new data have been submitted under this point. The notifier considers the previously submitted information to be acceptable to cover current requirements. No additional references were identified by the notifier from peer-reviewed open literature to be relevant for the mode of action of *Cydia pomonella* Granulovirus. Please refer to the "References relied on" section (chapter B.2.10) for a summary and an evaluation of the literature search.

B.2.2.2.1 Conclusion by the RMS (2019)

According to Commission Regulation (EU) No 283/2013 (Annex Part B Micro-organisms including viruses) four points need to be addressed in chapter B.2.2.2. These data requirements are outlined below followed by a short evaluation whether or not they have been adequately addressed by the notifier.

(i) "The principal mode of action shall be indicated. In connection with the mode of action it shall also be stated if the micro-organism produces a toxin with a residual effect on the target organism. In that case, the mode of action of this toxin shall be described."

(ii) "If relevant, information on the site of infection and mode of entry into the target organism and its susceptible stages shall be given. The results of any experimental studies must be reported."

As regards points (i) and (ii) RMS is of the opinion that the information provided may have been sufficient for the DAR but cannot be considered current state of knowledge for this renewal assessment report. While the infection process has shortly been outlined, i.e. oral intake → release of free virions in the midgut of the larvae → invasion of the midgut cells by fusion with the microvilli → histopathological effects in the larvae, the principal causes of the mode of action and intracellular entry of CpGV has not been indicated. RMS would like to point out that the information regarding these points has been taken from literature now twenty to forty years old. This does not mean that this literature is not relevant but the information provided can simply not be considered current state of knowledge. For example, the cited 1st edition of "The BioPesticide Manual" has been published in 1998. This manual has meanwhile several times been revised. The most recent edition (fifth edition) has been published in 2014 and an online version containing up-to-date information has been made available by the publisher. The baculovirus infection process has for example also well been reviewed in 2006 by Slack and Arif[6] including molecular mechanisms triggering the mode of action and mode of entry.

(iii) "It shall be stated by which way an uptake of the micro-organism, or its metabolites (especially toxins) may occur (e.g. contact, stomach, inhalation). It must also be stated whether or not the micro-organism or its metabolites are translocated in plants and, where relevant, how this translocation takes place."

RMS considers this data requirements as fulfilled.

[6] Slack and Arif, 2006, The Baculoviruses Occlusion-Derived Virus: Virion Structure and Function, Advances in Virus Research 69, 99-165, http://dx.doi.org/10.1016/S0065-3527(06)69003-9

(iv) "In case of pathogenic effect on the target organism, infective dose (the dose needed to cause infection with the intended effect on a target species) and transmissibility (possibility of spread of the micro-organism in the target population, but also from one target species to another (target) species) after application under the proposed condition of use shall be indicated."

Lethal doses, lethal concentrations, and lethal times have been provided, however RMS doubts that a 1% application rate, which has been suggested by Kienzle *et al.* (2003) to cause a considerable (but slow) mortality, has any meaning in practice. Slow larval mortality means considerable crop damage and therefore low efficacy. In addition, such low concentrations are not recommended in view of resistance management (J. A. Jehle, Julius Kühn Institut, Federal Research Centre for Cultivated Plants, Institute for Biological Control – personal communication). It is correct that dilutions of CpGV products still can initiate infections, since for all larvae a single occlusion body is enough to initiate an infection (LD_{50} = 1-10). The likelihood of infection, however, follows an Poisson distribution, thus, considering a population the infection rate is low and infection speed slow since several rounds of replications are needed to obtain a systemic infection (J. A. Jehle, personal communication). In spite of this the dosages recommended by the CpGV Task Force all appear appropriate (J. A. Jehle, personal communication).

Regarding CpGV's transmissibility in the target population the notifier presents information from a doctoral thesis published by Steineke in 2004, BVL no 2019090. However, the conclusions of the thesis and of the notifier are not necessarily the same. For example, while the notifer states that *"this study did neither clearly demonstrate nor prove vertical transmission of CpGV"*, the thesis' conclusion is that the finding *"suggests that CpGV is transmitted vertically via females. In terms of plant protection, CpGV could thus also affect subsequent codling moth generations in addition to the targeted generation. Moreover, vertical transmission could prove to play an important role in the persistence of CpGV in the field."* (see extended thesis abstract under http://nbn-resolving.de/urn:nbn:de:hebis:77-6595). Similarly, a reseach project conducted on behalf of the German Federal Environment Agency in 2002 concluded that CpGV *"is transmitted vertically via females, a finding that should also be corroborated by PCR analysis. This suggests that vertical transmission may also contribute to the persistence of a genetically modified virus in the field."* (Steineke and Jehle, 2002[7]). Thus, while the notifiers interpretation of Steinekes doctoral thesis is that vertical transmission may not have an impact on the transmissibility of CpGV, RMS's interpretation is that both the thesis and the research project do indeed suggest otherwise. However, according to J. A. Jehle (Julius Kühn Institut, Federal Research Centre for Cultivated Plants, Institute for Biological Control), Steineke's results were rather weak and could not be confirmed (personal communication). Moreover, there is hardly any reliable report on vertical transmission of baculoviruses in general (J. A. Jehle, personal communication).

Nonetheless, it would be important to resolve the issue of the viruses' transmissibility within the target population Notably, the notifier's search strategy may not have been sufficient to find recent literature dealing with this issue as neither "transmissibility" OR "transmission" OR"transmiss?" nor "vertical" OR "horizontal" or other search terms that could in any way be relevant here were included in the search strategy. However, according to J. A. Jehle there is indeed no recent literature on vertical or horizontal transmission of CpGV in codling moth field populations and a significant effect by horizontal and vertical transmission on population level of codling moth is not expected as otherwise weekly sprays of CpGV may not be necessary (J. A. Jehle, Julius Kühn Institut, Federal Research Centre for Cultivated Plants, Institute for Biological Control – personal communication).

[7] Steineke, S. B. and Jehle, J. A. (2002), Mathematical modelling of the population dynamics of genetically modified microorganisms using baculoviruses as example, Summary Report of the F&E project no. 298 89 418, UBA-Texte 63/02, https://www.umweltbundesamt.de/sites/default/files/medien/publikation/short/k2205.pdf

B.2.2.2.2 Cited references

Report KMA 2.2.2 – Copping, L.G., (1998). 1:36 *Cydia pomonella* granulosis virus. In: Copping, L.G. (ed.) (1998), The BioPesticide Manual. A world compendium, 1st edition, British Crop Protecion Council, pp. 60-61, Farnham, UK
Published report
BVL no 3714761

Abstract: The nomenclature of *Cydia pomonella* granulosis virus, its source, production, target pests and target crops, its biological activity, commercialization, application, product specifications, and compatibility, as well as its mammalian toxicity, environmental impact and non-target toxicity are shortly summarized.

Previous evaluation:	**in DAR (2007)**
Evaluation by the RMS (2019):	**Relevant for the DAR but possibly not reflecting the current state of knowledge. This manual has several times been revised. The most recent edition (fifth edition) has been published in 2014. Furthermore, an online version containing up-to-date information has been made available by the publisher (see links provided below).** https://www.bcpc.org/product/manual-of-biocontrol-agents-fifth-edition https://www.bcpc.org/product/manual-of-biocontrol-agents-online

Report KMA 2.2.2 – Tanada, Y., Leutenegger, R., (1968). Histopathology of a Granulosis-Virus Disease of the codling moth, *Carpocapsa pomonella*, Journal of Invertebrate Pathology, 10, 39-47
Published report
http://doi.org/10.1016/0022-2011(68)90261-9
BVL no 3714762

Abstract: The granulosis of the codling moth, *Carpocapsa pomonella*, is a polyorganotropic disease and produces pathologies in the fat body, hypodermis, tracheal matrix, and Malpighian tubules. Both free virus rods and capsules were observed in these organs with the electron microscope. The occurrence of infection in the Malpighian tubules suggests that the virus may be excreted through this organ. Laboratory tests indicated this possibility, but fecal contamination was not conclusively established. In addition to the virus rods and capsules found in the cells in an advanced stage of infection, there was a mass of coiled filaments that may be associated with the formation of the virus rods.

Previous evaluation:	**in DAR (2007)**
Evaluation by the RMS (2019):	**Relevant and reliable.**

Report KMA 2.2.2 – Tanada, Y., Kaya, H.K. (1993), DNA-Viral Infections: Baculoviridae, In: Vega, F. and Kaya, H.K. (ed.), Insect Pathology, pp. 171-244. London: Academic Press Inc.
Published report
BVL no 3714763

Abstract: The word *virus* is derived from Latin and means a slimy liquid, poison, or stench.The early definition of a virus was based on submicroscopic size and obligate pathogenicity. More recently, the definitions attempted to convey two qualities of the virus: (1) possession of its own genetic material, which inside the host cell behaved as part of the cell, and (2) presence of a submicroscopic infective stage, the virion, which served as the vehicle for introducing the viral genome into a cell (Lwoff and Tournier 1971). These definitions, however, did not adequately separate viruses from other minute parasitic procaryotes, such as rickettsiae, mycoplasma, and chlamydia. Matthews (1991) has thoroughly discussed the characterization of these procaryotes and their differences from the virus. He defined a virus as follows:

A virus is a set of one or more nucleic acid template molecules, normally encased in a protective coat or coats of protein or lipoprotein, that is able to organize its own replication only within suitable host cells. Within such cells, virus replication is (i) dependent on the host's protein-synthesizing machinery, (ii) organized from pools of the required materials rather than by binary fission, (iii) located at sites that are not separated from the host cell contents by a lipoprotein bilayer membrane, and (iv) continually giving rise to variants through various kinds of change in the viral nucleic acid.

The virus must normally be transmissible and cause disease in a host. Viral diseases are one of the most widely investigated infections in insects. These studies have resulted because of the extensive basic and applied interests in viruses and from the development of elaborate and complex equipment, including the sophisticated techniques in biochemistry, serology, pathology, tissue culture, and recombinant DNA technology. With these advances, applied insect virology has extended beyond pest control into the field of genetic engineering, where the virus serves a vector for the expression of foreign genes to produce biochemically and pharmaceutically important products. The accomplishments in genetic engineering are due primarily to the availability of invertebrate cell lines. Up to 1989, cell lines have been formed from 55 species of invertebrates in seven orders within Arthropoda and with most of them from Lepidopter and Diptera (Hink and Hall 1989).

Previous evaluation:	**in DAR (2007)**
Evaluation by the RMS (2019):	**Relevant and reliable, but possibly not reflecting the current state of knowledge. A revised edition (2nd edition) of this book was published in 2012. Chapter 4 of the 2nd edition, i.e. "Baculoviruses and Other Occluded Insect Viruses"by Harrison∗and Hoover would perhaps be the more appropriate source (see links provided below)** https://www.elsevier.com/books/insect-pathology/vega/978-0-12-384984-7 https://doi.org/10.1016/B978-0-12-384984-7.00004-X

Report KMA 2.2.2 – Bilimoria, S.L. (1986), Taxonomy and Identification of Baculoviruses, In: Granados, R.R and Federici, B.A. (eds.), The Biology of Baculoviruses, Volume 1: Biological Properties and Molecular Biology, pp. 37-59. CRC Press, Boca Raton
Published report
BVL no 3689461

Abstract: This chapter describes the basic properties of the major subgroups of baculoviruses and reviews the most useful and practical approaches for their identification and classification. Special emphasis is placed on the impact of recent technological developments on baculovirus taxonomy. Several previous reviews have addressed the question of the classification and identification of baculoviruses, and the reader may wish to consult these for earlier developments about which detailed information may not be provided in the following account. Longworth recently discussed current problems in insect virus taxonomy.

Previous evaluation:	**in DAR (2007)**
Evaluation by the RMS (2019):	**Possibly relevant, however, only in the context being used. Otherwise this review is really dated, especially with reference to taxonomy. Regarding taxonomy the right source would be: Herniou, E. A., Arif, B. M., Becnel, J. J., Blissard, G. W., Bonning, B., Harrison, R., Jehle, J. A., Theilmann, D. A. and Vlak, J. M., Baculoviridae. In: King, A. M. Q., Adams, M. J., Carstens, E. B., Lefkowitz, E. J. (editors). *Virus Taxonomy*. Oxford: Elsevier; 2011. pp. 163–174.**

Report KMA 2.2.2 – Steineke, S.B. (2004), Populationsdynamik des *Cydia pomonella* Granulovirus, Dissertation zur Erlangung des Grades "Doktor der Naturwissenschaften" am Fachbereich Biologie der Johannes Gutenberg-Universität in Mainz, pp. 134
Published report
BVL no 2019090

Abstract: The *Cydia pomonella* Granulovirus (CpGV, Fam. Baculoviridae), an extremely virulent and highly specific pathogen, has been registered for the control of the codling moth (*Cydia pomonella*) in Germany and other countries of the EU. It infects the larval stages of its host and does not harm non target organisms. Past research on CpGV addressed questions relevant to its production and application as pest control agent. However, 20 years after the first registration, it remains unclear whether CpGV can establish itself in the environment. As part of this project, various parameters were analysed and quantified to aid in describing CpGV's population dynamics. The studied parameters included virulence, virus yield, horizontal and vertical transmission, inactivation rate and the infection rate of late instars. The quantified parameters were then integrated into a mathematical model along with data found in the relevant literature.

For the extended abstract please refer to:
https://publications.ub.uni-mainz.de/theses/frontdoor.php?source_opus=659

Previous evaluation:	**in DAR (2007)**
Evaluation by the RMS (2019):	**Relevant and reliable.**

Report KMA 2.2.2 – Steineke, S.B. and Jehle, J. A. (2004), Investigating the horizontal transmission of the *Cydia pomonella* granulovirus (CpGV) in a model system, Biological Control, 30, 538-545
Published report
https://doi.org/10.1016/j.biocontrol.2004.02.010
BVL no 2019092

Abstract: Repeated applications of *Cydia pomonella* granulovirus (CpGV) can effectively control the codling moth (CM) in apple orchards. However, it is still unknown whether horizontal transmission of the virus from infected to uninfected larvae contributes to the efficacy of the virus insecticide. Horizontal transmission of CpGV was assayed using detached apples. In experiments using artificially applied virus dots on the apple's surface or infected CM larvae as virus inoculum, it was found that the likelihood of infection of healthy CM larvae relied mainly on the larval behavior. The amount of virus inoculum, either applied artificially or produced by the infected larvae, impacted the infection rate only to a small degree. In the experiments, CM larvae exhibited a strong preference in entry sites, increasing the chance for horizontal transmission. Depending on the experimental design, horizontal transmission rates of about 40% were observed in laboratory assays.

	Suggested by RMS
Evaluation by the RMS (2019):	**Relevant and reliable.**

Report KMA 2.2.2 – Kienzle, J., Schulz, C, Zebitz, C.P.W., Huber, J. (2003), Persistence of the Biological Effect of Codling Moth Granulovirus in the Orchard - Preliminary Field Trials, In: Papierok, B. (ed.) IOBC/WPRS Bulletin Vol. 26 (1), Working Group "Insect Pathogens and Insect Parasitic Nematodes", Proceedings of the 8th European Meeting "Entomopathogens and Insect Parasitic Nematodes: Current Research and Perspectives in Pest Biocontrol" at Athens (Greece), 29 May - 2 June 2001, pp. 245-248. Published report BVL no 3682783
Abstract: In 2000 and 2001, in a field trial, the persistence of the biological effect of codling moth granulovirus (CpGV) was investigated. With a single treatment at full concentration of CpGV (MADEX 3, 100 ml/ha) a considerable reduction of CM population was achieved over the whole vegetation period. This may indicate, that over a considerable period of time after a treatment a biological effect of CpGV sufficient for an increased mortality of the larvae was present in the orchard. However, the onset of mortality was not fast enough to protect the fruit from damage. Further research has to be done to gain more experience in handling this effect. It could be very important for the reduction of the number of treatments in organic apple growing.

Previous evaluation:	**in DAR (2007)**
Evaluation by the RMS (2019):	**Relevant and reliable, however, it should be noted that the publication copy provided is not the one that has been cited. The copy provided (which is similar in content to the cited publication) has already been published in 2002 and comes from a different source, i.e.:** Kienzle, J. Schulz, C., Zebitz, C.P.W., Huber, J. (2002). Persistence of the biological effect of codling moth granulovirus in the orchard - preliminary field trials, 10th International Conference on Cultivation Technique and Phytopathological Problems in Organic-Fruit-Growing and Viticulture, Proceedings to the conference from 4th to7th February 2002 at Staatliche Lehr- und Versuchsanstalt für Wein- und Obstbau Weinsberg, pp. 187-191

B.2.3 Host specificity range and effects on species other than the target harmful organism

The following information, highlighted in grey, is derived from the Draft Assessment Report Volume 3, Annex B-2, Point B.2.4.

Baculoviruses have been found only in arthropods, particularly in members of Lepidoptera (mainly), Hymenoptera (few) and Diptera (very few). No member of this family is known to infest vertebrates or plants.

Granuloviruses are reported only from lepidopteran hosts and are, in general, even more selective than Nucleopolyhedroviruses. The host range of the Granuloviruses is mostly restricted to a single species (OECD, 2002, BVL no 3682775). Reports of successful and unsuccessful attempts to cross-transmit CpGV to alternative hosts are summarised in Table B.2.3-1 (Gröner, 1986 and references therein, BVL no 3682784):

Table B.2.3-1: CpGV isolated from *Cydia pomonella* and attempted to cross-transmit to alternative lepidopteran hosts

Family	Species	Result
Tortricidae	*Archips podanus*	-

	Archips sorbianus	-
	Adoxophyes orana	-
	Choristoneura muriana	-
	Enarmonia formosana	-
	Grapholita funebrana	-
	Grapholita molesta	+
	Hedya nubiferana	-
	Laspeyresia nigricana	+
	Pandemis heparana	-
	Rhyacionia buoliana	+
	Zeiraphera diniana	-
Geometridae	*Operophtera brumata*	-
Noctuidae	*Agrotis segetum*	-
	Autographa gamma	-
	Heliothis zea	-
	Mamestra brassicae	-
Plutellidae	*Plutella xylostella*	-
Pyralidae	*Amyelois transitella*	-
Saturniidae	*Antheraea pernyi*	-

successful attempts of cross-transmission (+) are in bold, (-) unsuccessful attempt of cross-transmission

In conclusion of these experiments, CpGV was only able to infect three additional hosts, namely the oriental fruit moth Grapholita molesta, the pea moth Laspeyresia (*Cydia) nigricana,* and the pine shoot moth *Rhyacionia buoliana*. CpGV-M could not be transmitted to nine other tortricid species including, *Adoxophyes orana* and *Grapholita funebrana*. No transmission to species not belonging to the Tortricidae was observed. This shows that CpGV is restricted in its infectivity to very few hosts of the Tortricidae family only.

Fritsch *et al.* (1990, BVL no 2390234) tested *Cydia pomonella* GV and *Cryptophlebia leucotreta* GV by bioassays with neonate larvae. The virulence of each virus to its host, CpGV to *C. pomonella* and CrleGV to *C. leucotreta* (Lepidoptera: Tortricidae), were similar. However CpGV was shown to be cross-infectious to the false codling moth, *C. leucotreta*, but with a virulence 1000 times less than the specific granulovirus, while the CrleGV is not infectious to *C. pomonella* (see Table B.2.3-2).

Table B.2.3-2: Biological activity of *Cryptophlebia leucotreta* GV (CrleGV) and *Cydia pomonella* GV (CpGV) determined in bioassays with neonate larvae

Target species	**LC_{50} (g/mL diet)**	
	CrleGV	**CpGV**
Cryptophlebia leucotreta	2.37×10^{1}	3.28×10^{4}
Cydia pomonella	$> 1 \times 10^{9}$	2.84×10^{1}

New data 2016/2017

CpGV-V14

The host spectrum for CpGV-V14 was determined by Züger (2011a, BVL no 3714765) using *Spodoptera littoralis* (Noctuidae), *Adoxophyes orana*, *Cryptophlebia leucotreta* and *Grapholita molesta* (all Tortricidae). Even at dose rates far higher than required to kill *Cydia pomonella* larvae, no effect was observed for *S. littoralis*, *A. orana* and *G. molesta*. The only species that showed a high mortality rate was *Cr. leucotreta*, which is very closely related to *C. pomonella*.

Within the host *C. pomonella*, CpGV-V14 was tested in larvae that were susceptible for CpGV-M and also in larvae that derived from CpGV-M resistant field populations (Züger, 2011b, BVL no 3714766). In both experiments, CpGV-V14 achieved similar results. Thus, this particular isolate can be considered as a potentially effective agent against codling moth populations possessing a similar kind of resistance against CpGV-M.

Taken together, CpGV-V14 is restricted in its infectivity to very few hosts (*C. pomonella* and to certain extent to *Cr. leucotreta*) of the Tortricidae family only. Its very high host-specificity is especially important for assessing the side-effects on beneficial arthropods and other non-target organisms. No differences in effects on non-host arthropods, other animals including vertebrates, plants, or microorganisms are expected between CpGV-V14 and CpGV-M.

CpGV-V45

The host spectrum for the isolate CpGV-V45 was determined by Züger (2017a, BVL no 3714813) using the noctuid species *Spodoptera littoralis*, *S. exigua* and *Helicoverpa armigera* (Noctuidae) and the tortricid species *Adoxophyes orana* (Tortricidae). Even at dose rates far higher than required to kill *Cydia pomonella* or *Grapholita molesta* larvae, no effect was observed for *S. littoralis*, *S. exigua*, *H. armigera* and *A. orana*.

Within the host *C. pomonella*, CpGV-V45 was tested in larvae that were susceptible for CpGV-M, in larvae that derived from CpGV-M resistant field populations and in *Grapholita molesta* larvae (Züger, 2017b, BVL no 3714814). In all experiments, CpGV-V45 achieved similar results leading to high mortalities in the tested animals. The responses achieved in *Grapholita molesta* exceeded those of similar CpGV isolates already been in use. In addition, this particular isolate can be considered as a potentially effective agent against codling moth populations possessing a similar kind of resistance against CpGV-M.

Taken together, CpGV-V45 is restricted in its infectivity to very few hosts (*C. pomonella* and *G. molesta*) of the Tortricidae family only. Its very high host-specificity is especially important for assessing the side-effects on beneficial arthropods and other non-target organisms. No differences in effects on non-host arthropods, other animals including vertebrates, plants, or microorganisms are expected between CpGV-V45 and CpGV-M.

No additional references were identified by the notifier from peer-reviewed open literature to be relevant for the data point “Host specificity range and effects on species other than the target harmful organism”. Please refer to the “References relied on” section (chapter B.2.10) for a summary and an evaluation of the literature search.

B.2.3.1 Conclusion by the RMS (2019)

According to Commission Regulation (EU) No 283/2013 (Annex Part B Micro-organisms including viruses) two points need to be addressed in chapter B.2.3. These data requirements are outlined below followed by a short evaluation whether or not they have been adequately addressed by the notifier.

(i) "Any available information on the effects on non-target organisms within the area to which the micro-organism may spread shall be given. The occurrence of non-target organisms being either closely related to the target species or being especially exposed shall be indicated."

While some information concerning these points has been presented, RMS would like to note that the information regarding the host specificity range is mainly based on the review by Gröner (1986, BVL no 3682784). Though being highly valuable, this review has been published more than 30 years ago and, what is more, the information summarized in Table B.2.3-1 (which has been taken from this review) is primarily based on literature published in 1978. Thus, the information provided is now about 40 years old. However, according to J. A. Jehle, there is indeed no new evidence that the host range of CpGV is broader than reviewed by Gröner (1986). Considering that nearly all known baculoviruses have been isolated from Lepidoptera and given the higly restricted host range within Tortricidae and given the co-evolution of single baculoviruses and their hosts it is fully unlikely that CpGV infects non-Tortricid species (J. A. Jehle, Julius Kühn Institut, Federal Research Centre for Cultivated Plants, Institute for Biological Control – personal communication).

(ii) "Any experience of the toxic effect of the active substance or its metabolic products on humans or animals, of whether the organism is capable of colonising or invading humans or animals (including immunosuppressed individuals) and whether it is pathogenic shall be stated. Any experience of whether the active substance or its products may irritate skin, eyes or respiratory organs of humans or animals and whether it is allergenic in contact with skin or when inhaled shall be stated."

This point has not been addressed in this chapter, however, it has been dealt with separately in the introductory part of Section B.2. "Biological properties of the micro-organism" where the notifier explicitly states: *"CpGV is not supposed to have any harmful effects on organisms not belonging to the family of Tortricidae. With regard to environmental safety it is important to note that CpGV and the whole group of baculoviruses are naturally present in the environment. The experience that baculoviruses present no risk to mammals and men has been confirmed by numerous studies. The family of baculoviruses is regarded to be safe for humans and vertebrates confirmed by the inclusion of this virus family in the list of "Qualified Presumption of Safety" published by EFSA. Therefore, their application in pest control means only a fluctuation of the virus titre in the biotope of the pest insect. CpGV and the whole family of baculoviruses are not related to any animal (other than arthropods) or plant pathogen and it does not produce any metabolite. For these reasons, no harmful effects from CpGV on humans, other vertebrates, other non-target organisms or the environment are expected."* This statement should be repeated in chapter B.2.3 and supplemented with the necessary references.

B.2.3.2 Cited references

Report KMA 2.3 – OECD (2002), Consensus Document on Information used in the Assessment of Environmental Applications involving *Baculovirus* (ENV/JM/MONO(2002)1), Series on Harmonization of Regulatory Oversight in Biotechnology, No.20, OECD Environment Directorate, Paris. (Available on the Biotrack website at http://www.oecd.org/biotrack/)
Published report
BVL no 2682775

Abstract: The OECD's Working Group on Harmonization of Regulatory Oversight in Biotechnology decided at its first session, in June 1995, to focus its work on the development of consensus documents which are mutually acceptable among Member countries. These consensus documents contain information for use during the regulatory assessment of a particular product. This document contains general information on baculoviruses such as organism characteristics, behavior in the environment, their history of use and interactions, as well as environmental safety considerations. Germany served as lead country in the preparation of this document. It has been revised on a number of occasions based on the input from other Member countries. It is intended for use by regulatory authorities and

others who have responsibility for assessments and by those who are actively involved with genetic improvement and intensive management of the genus.

Previous evaluation: in DAR (2007)
Evaluation by the RMS (2019): Relevant and reliable.

Report KMA 2.3 – Gröner, A. (1986), Specificity and Safety of Baculoviruses, In: Granados, R.R and Federici, B.A. (eds.), The Biology of Baculoviruses, Volume 1: Biological Properties and Molecular Biology, pp. 177-201. CRC Press, Boca Raton
Published report
BVL no 3682784

Abstract: Increasing public concern regarding contamination of the environment in recent years has resulted in a critical reevaluation of the methods used in plant protection. The public is especially concerned about the effects that chemical pesticides have on various ecosystems. The necessity for protecting plants against insect populations, which must be kept below an economic threshold through the application of chemical insecticides, has caused, to some degree, an environmental hazard by contamination with residues, has increased the resistance of pest insects, and has created new insect pests through the elimination of natural enemies that normally keep these insects below the economic threshold. This situations has resulted in a search for alternative agents for plant protection and an increased demand for biological pest control. It is currently hoped that biological agents which are safer and nonpolluting can be developed for pest control. Importantly, the occurrence of natural epizootics caused by insect pathogens, particularly baculoviruses, in insect pest populations in field crops and forests has demonstrated the potential for using these agents in pest-management programs. However, even though these agents occur naturally, it must be demonstrated that they are environmentally safe and free of hazards for man, domestic animals, and wildlife before they can be used in pest-control programs. In Table 1, the host range members of viral families infecting invertebrates is shown. Baculoviruses have been found only in invertebrates; no member of this family is known to infect vertebrates or plants. In this chapter, the evidence regarding the limitation of the host range of baculoviruses to certain invertebrates and their safety for nontarget organisms is reviewed.

Previous evaluation: in DAR (2007)
Evaluation by the RMS (2019) Relevant and reliable. Although this reference is has been published more than 30 years ago there is no new evidence that the host range of CpGV is broader than than that reviewed.

Report KMA 2.3 – Fritsch, E., Huber, J., Backhaus, H. (1990), CpGV as a Tool in the Risk Assessment of Genetically Engineered Baculoviruses, In: Vth International Colloquium on Invertebrate Pathology and Microbial Control, incorporating the XXIIIrd Annual Meeting of the Society for Invertebrate Pathology, Proceedings and Abstracts, Adelaide, Australia, 20-24 August 1990. pp. 439-443
Published report
BVL no 2390234

Abstract: Before genetically modified baculoviruses are considered for a deliberate release into the environment thorough understanding of the consequences of such an action is needed. The safety of constructed recombinant insect pathogens has to be examined carefully. Particular attention must be paid to the possible risk of genetic exchange of engineered characteristics with natural occurring organisms, therby creating unforeseen hazards. The aim of this study is to examine and quantify the likelihood of recombination of granulosis viruses with regard to the transfer of genetic characteristics. It is intended to study the genetic exchange of these pathogens *in vivo* in living host larvae. Therefore larvae shall be coinfected *per os* by contamination of diet with a mixture of granulosis viruses, since this is the natural way of virus infection in the insect. As representative organisms the viruses of two closely related tortricids are chosen – the granulosis virus of the codling moth *Cydia pomonella* (CpGV) and that of the false codling moth *Cryptophlebia leucotreta* (ClGV). Both granulosis viruses

are highly pathogenic for their homologous hosts. Bioassays with neonate larvae showed that the virulence of the ClGV for *C. leucotreta* is similar to that of the CpGV for *C. pomonella*. Furthermore the CpGV was found to be crossinfectious for larvae of the false codling moth, but it is about 1000 times less virulent that the specific granulosis virus. The ClGV, on the other hand, is not infective for *Cydia pomonella*, even at high virus concentrations up to 10^9 granules/ml diet. The ClGV and the CpGV have been characterized not only biologically but biochemically as well. In SDS-PAGE a high degree of similarity in the protein patterns of the pathogens was obtained. Analysis of the viral genome by restriction enzymes showed that the CpGV and ClGV differ substantially in their characteristic DNA profiles. The restriction sites as markers are helpful to identify viral progeny after mixed infections with the ClGV and CpGV in host larvae.

Previous evaluation: in DAR (2007)
Evaluation by the RMS (2019): Relevant and reliable.

Report KMA 2.3/01 – Züger, M. (2011a), Host range CpGV isolate ABC-V14 I 2011
unpublished
BVL no 3714765

Abstract: CpGV isolate ABC-V14 is a newly developed CpGV isolate, which shows good efficacy against sensitive as well as resistant codling moth (*Cydia pomonella*). The aim of this trial was to determine the host range of CpGV isolate ABC-V14. In spite of a high overdosage, CpGV isolate ABC-V14 showed no effect on *Spodoptera littoralis* and *Adoxophyes orana* and *Grapholita molesta*. The only exception is *Cryptophlebia leucotreta*, a closely related species to the actual target organism (codling moth).

Previous evaluation: Submitted for the purpose of renewal
Evaluation by the RMS (2019): Relevant.

Report KMA 2.3/02 – Züger, M. (2011b), Bioassay with CpGV isolate ABC V14 against CpGV-sensitive and CpGV-resistant larvae of *Cydia pomonella*
unpublished
BVL no 3714766

Abstract: The activity of the CpGV isolate ABC V14 was investigated in bioassays on sensitive and resistant codling moth larvae (CM) (*Cydia pomonella*). The CpGV isolate ABC-V14 showed good efficacy on both CM strains.

Previous evaluation: Submitted for the purpose of renewal
Evaluation by the RMS (2019): Relevant.

Report KMA 2.3/03 – Züger, M. (2017a), AW: Host range CpGV isolate ABC-V45 I 2017
unpublished
BVL no 3714813

Abstract: CpGV isolate ABC-V45 is a newly developed CpGV isolate, which shows good efficacy against sensitive as well as resistant codling moth (*Cydia pomonella*). Furthermore, it can be used to control oriental fruit moth. The aim of this trial was to determine the host range of CpGV isolate ABC-V14. In spite of a high overdosage of 100,000 OB's per gram diet, CpGV isolate ABC-V45 showed no effect on *Spodoptera littoralis*, *Adoxophyes orana*, *Helicoverpa armigera* and *Spodoptera exigua*.

Previous evaluation: Submitted for the purpose of renewal
Evaluation by the RMS (2019): Relevant.

Report KMA 2.3/04 – Züger, M. (2017b), AW: Bioassay with CpGV isolate ABC-V45 against *Grapholita molesta*, CpGV sensitive and resistant larvae of *Cydia pomonella* I 2017 unpublished BVL no 3714814
Abstract: The activity of the CpGV isolate ABC-V45 was investigated in bioassays on three lepidopteran populations. ABC-V45 showed good efficacy on oriental fruit moth (OFM, *Grapholita molesta*), sensitive and resistant codling moth larvae (CM, *Cydia pomonella*).
Previous evaluation: Submitted for the purpose of renewal **Evaluation by the RMS (2019): Relevant.**

B.2.4 Development stages/life cycle of the micro-organism

The following information, highlighted in grey, is derived from the Draft Assessment Report Volume 3, Annex B-2, Point B.2.1.4.

The natural route of infection is the peroral ingestion of viral occlusion bodies by larvae. In the alkaline environment of the midgut (pH > 9.5), the occlusion bodies dissolve rapidly and occlusion-derived virions (ODV's) are released (Evans and Harrap, 1982; OECD, 2002, BVL no 3682785). There is evidence that the dissolution of the occlusion body matrix might be facilitated by an insect derived alkaline protease which is associated with the occlusion body matrix. The ODV`s pass through the peritrophic membrane (PM), a proteinaceous-chitinaceous layer which is secreted by the midgut cells to protect the midgut epithelium from direct contact with ingested material. After attachment to the microvilli of the midgut epithelium, the nucleocapsids enter the cell lumen either via fusion of the virion envelope with the epithelial membrane or by viropexis. The nucleocapsids are transported, most likely under involvement of the cellular microtubular structures, to the nucleus and become uncoated at the nuclear pore or within the nucleus where the viral DNA is released and DNA expression and replication is initiated (OECD, 2002). Initial replication produces non-occluded virus particles to hasten the invasion of the host insect (Copping, 1998, BVL no 3682777).

Hess and Falcon (1987, BVL no 2019102) studied the replication cycle of the Granulovirus of *Cydia pomonella* at the cellular and tissue level. Membrane-like complexes were observed forming within the remnants of the nucleolus in the cytoplasm of infected cells. Differences in cell polarity relative to the sites of virus entry assembly and budding as well as the differences in the temporal aspects of replication were observed between midgut, fat body, and epidermal cells. The progressive spread of virus throughout larval tissues was studied at 24, 32, 48, 56, and 72 hour post infection.

By exocytosis the newly formed virions get to the hemolymph and from there into various tissues of the organism. In the cells of the fat body, hypodermis, Malphigian tubules and tracheal matrix, free virus rods, with and without developmental membrane, partly encapsulated rods and capsules have been observed already three days after feeding (Bilimoria, 1986, BVL no 3689461). After cell lysis a large number of occluded CpGV will be set free which are able to infest new hosts.

Gene expression

Baculoviruses do not possess a metabolism on their own and require the cell apparatus of their hosts for replication and multiplication. The infection cycle is the only stage in which baculovirus genes are actively translated into proteins and it is divided roughly into three stages according to the promotor elements that administer gene transcription. In the early stage of infection the RNA polymerase II of the host cell is used to transcribe early baculovirus genes, while with beginning of the late stage baculoviruses encode for their own RNA polymerase (Huh and Weaver, 1990, BVL no 3714815). Baculovirus genes are expressed in a transcriptional cascade in which each successive phase is dependent on the expression of genes during the previous phase. The virus-specific RNA polymerase has a unique subunit

composition (Kool et al., 1995 and references therein, BVL no 2019110). This polymerase is resistant to α-amanitin (an RNA Pol II toxin) and tagetitoxin (an RNA Pol III toxin) and initiates transcription from within a 5 bp late promoter element with the sequence A/G/T TAAG. All baculoviruses further encode their own DNA polymerase during the late and very late stage of infection and the hosts own DNA polymerase is blocked at that part of infection.

Late gene expression is dependent on viral DNA replication and is not observed when DNA replication is inhibited (e.g. by aphidicolin or cytosine arabinoside). After the onset of DNA replication, most late genes are actively transcribed but the levels of expression decline at later times. However, expression of the polyhedrin gene which encodes the major occlusion body protein is initially delayed but subsequently reaches extremely high levels very late in infection. In addition, p10, which encodes a small poorly conserved protein that may be involved in occlusion body formation or cell lysis is abundantly expressed at both late and very late times post-infection. Polyhedrin and p10 have been termed 'very late genes' (Kool *et al.*, 1995).

A key characteristic of the very late stage are the production of envelope proteins and occlusion body proteins (e.g. Granulin). The embedded virions can be considered as a dormant stage of baculoviruses. These occlusion bodies endure hazardous environmental conditions such as exposure to UV radiation, draught or excess humidity as well as enzymatic degradation (Jaques, 1977, BVL no 3728861; Evans and Harrap, 1982).

Survival time

The occlusion body protects the virion and makes it quite stable at moderate and low temperatures, in soil and water, and resistant to various chemicals. Because of their resistance to environmental conditions occlusion bodies will retain their infectivity for long periods, for example 20 years stored as dry powders or in flame-sealed gloss tubes (Evans and Harrap, 1982 and references therein). Viruses stored as intact occlusion bodies may retain activity for several years in storage in the dark at 4°C (Jaques, 1977 and references therein) whereas virions in hemolymph or released from occlusion bodies retain activity for much shorter periods. Because of the relative instability of virions, activity of free virions is not a major consideration in the efficacy of occluded viruses applied in the field (Jaques, 1977).

New data 2016/2017

One new study, Huh and Weaver (1990) has been submitted by the notifer, however as this study does not provide information that had not already been presented in the DAR, RMS included this information into the information derived from the DAR. In general, the notifier considers the previously submitted information to be acceptable to cover current requirements. No additional references were identified by the notifier from peer-reviewed open literature to be relevant in regard to developmental stages/ life cycle of CpGV. Please refer to the "References relied on" section (chapter B.2.10) for a summary and an evaluation of the literature search.

B.2.4.1 Conclusion by the RMS (2019)

According to Commission Regulation (EU) No 283/2013 (Annex Part B Micro-organisms including viruses) four points need to be addressed in chapter B.2.4. These data requirements are outlined below followed by a short evaluation whether or not they have been adequately addressed by the notifier.

(i) "Information on the life cycle of the micro-organism, described symbiosis, parasitism, competitors, predators, etc., including host organisms, as well as vectors for viruses, must be presented."

While an infectious cycle has been described it should be noted that this description refers to the replication of baculoviruses in general. Notably, the description has mainly been taken from the OECD Consensus Document (2002), however, the Consensus Document points out that its description refers to the replication of the *Autographa californica* multinucleocapsid nuclear polyhedrosis

virus (AcMNPV) which serves as a model for NPV and GV replication in Lepidoptera. In contrast, this information was not provided by the notifier. Similarly, the review by Evans and Harrap (1982) refers to entomopathogenic viruses – again in general. Yet, Hess and Falcon (1987) – which have also been cited by the notifier – state that some exceptions to the basic replicative sequence of granulosis viruses do occur in CpGV, however, these exceptions have not been summarized by the notifier.

Furthermore, the notifier presents information on gene expression in baculoviruses which – though not being stated – is also based on research with AcMNPV (Huh and Weaver, 1990; Kool *et al.*, 1995). While gene expression in CpGV may indeed be similar or even identical to expression in AcMNPV – and all evidence indeed suggests so[8] – this information is not necessarily relevant unless molecular events are generally included in the description of the virus's life cycle.

Relevant information dealing with gene expression in CpGV can be found in the following literature:

- Herniou, E. A., Arif, B. M., Becnel, J. J., Blissard, G. W., Bonning, B., Harrison, R., Jehle, J. A., Theilmann, D. A. and Vlak, J. M. (2011). Baculoviridae. In: King, A. M. Q., Adams, M. J., Carstens, E. B., Lefkowitz, E. J. (editors). *Virus Taxonomy*. Oxford: Elsevier; 2011. pp. 163-174.

- Luque, T., Finch, R., Crook, N., O'Reilly, D., Winstanley, D. (2001). The complete sequence of the *Cydia pomonella* granulovirus genome, Journal of General Virology, 82, 2531–2547, https://dx.doi.org/doi:10.1099/0022-1317-82-10-2531

- Wennmann, J., Radtke, P., Eberle, K. E., Gueli Alletti, G., Jehle, J. A. (2017). Deciphering single nucleotide polymorphisms and evolutionary trends in isolates of the *Cydia pomonella* granulovirus. Viruses 9(8), 227, https://dx.doi.org/10.3390/v9080227

- Eberle, K. (2010). Novel isolates of *Cydia pomonella* granulovirus (CpGV): deciphering the molecular mechanism for overcoming CpGV resistance in codling moth (*Cydia pomonella*), PhD thesis (Dissertation zur Erlangung des Grades Doktor der Naturwissenschaften am Fachbereich Biologie der Johannes Gutenberg-Universität Mainz), pp. 181, http://nbn-resolving.de/urn:nbn:de:hebis:77-24208

- Elemnofy, W. (2008). Analysing the possible influence of transposon TCl4.7 insertion on the function of the genome of *Cydia pomonella* granulovirus, PhD thesis (Dissertation zur Erlangung des Grades Doktor der Naturwissenschaften am Fachbereich Biologie der Johannes Gutenberg-Universität Mainz), pp. 126, http://nbn-resolving.de/urn:nbn:de:hebis:77-18387

As regards the survival time of CpGV, the information provided concerns baculoviruses in general. However, a recent publication deals explicitly with the effect of temperature on long-term storage of *Cydia pomonella* Granulovirus and should be included (Sauer and Jehle, 2017, Effect of temperature on long-term storage of *Cydia pomonella* Granulovirus (CpGV-M), IOBC-WPRS Bulletin Vol. 129, pp. 184-188.

[8] According to J. A. Jehle (Julius Kühn Institut, Federal Research Centre for Cultivated Plants, Institute for Biological Control – personal communication) the presence of the same promoter motives for early promoters (transcribed by host RNA polymerases) and late promoters (transcribed by viral RNA Polymerase) in all baculoviruses and the presence of the same homologs encoding the viral RNA polymerase subunits in all baculovirus genomes (so-called baculovirus core genes) highly supports a universal gene transcription and expression machinery that is very similar in and specific to all baculoviruses. Therefore, gene transcription and expression of AcMNPV is indeed a model for all baculoviruses. This assumption is coroborated by the identification of baculovirus core genes in CpGV, identification of early and late promoter motifs, and expression studies of selected CpGV genes.

In conclusion, it occurs to RMS that the description of the replicative sequence of CpGV is rather general than specific and does much likely not reflect the current state of knowledge.

The remaining information necessary for point (i) of the data requirements, i.e. information on described symbioses, parasitism, competitors, predators, etc., including host organisms, as well as vectors for viruses is obvious and has been provided elsewhere in this section.

(ii) "The generation time and the type of reproduction of the micro-organism must be stated."

No statement has been made regarding this point. Concerning the type of reproduction it is obvious that multiplication is that of viruses in general, i.e. reproduction occurs intracellularly by making use of the host cell resources. However, concerning the generation time of CpGV some information would be necessary as a straight and timely balanced series of proliferation events can be assumed from the oral ingestion of the occlusion body by the host larva to larval liquefaction and release of the virus.

(iii) "Information on the occurrence of resting stages and their survival time, their virulence and infection potential must be provided."

Some basic information regarding this point has been provided, however regarding virulence and infection potential no explicit statement has been made. Nevertheless, it is obvious that CpGV is extremely virulent and highly infectious to *C. pomonella* and *G. molesta* larvae.

(iv) "The potential of the micro-organism to produce metabolites, including toxins that are of concern for human health and/or the environment, in its different development stages after the release, must be indicated."

No statement has been made regarding this point, however, as pointed out before by RMS it has been dealt with separately in the introductory part of Section B.2. "Biological properties of the micro-organism" where the notifier explicitly states: *"CpGV is not supposed to have any harmful effects on organisms not belonging to the family of Tortricidae. With regard to environmental safety it is important to note that CpGV and the whole group of baculoviruses are naturally present in the environment. The experience that baculoviruses present no risk to mammals and men has been confirmed by numerous studies. The family of baculoviruses is regarded to be safe for humans and vertebrates confirmed by the inclusion of this virus family in the list of "Qualified Presumption of Safety" published by EFSA. Therefore, their application in pest control means only a fluctuation of the virus titre in the biotope of the pest insect. CpGV and the whole family of baculoviruses are not related to any animal (other than arthropods) or plant pathogen and it does not produce any metabolite. For these reasons, no harmful effects from CpGV on humans, other vertebrates, other non-target organisms or the environment are expected."* This statement should be repeated in chapter B.2.4 and supplemented with the necessary references.

The notifiers conclusion that no additional references were identified from peer-reviewed open literature to be relevant concerning the developmental stages/ life cycle of CpGV is evident as the literature search strategy was missing several specific search terms that would have been necessary for chapter B.2.4 such as "development", "reproduction", "replication", "proliferation", "life cycle", "infectious cycle", "reproductive cycle" or other terms that could in any way be relevant for this chapter. Furthermore, the notifier's introductory statement that CpGV does not produce any metabolites has not been backed up by a recent reference. Moreover, neither "metabolite" nor "toxin" were included as specific search terms in the search strategy. However, a literature search covering the last ten years would be necessary to maintain the previous evidence that no metabolites and toxins are produced by CpGV.

B.2.4.2 Cited references

Report KMA 2.4 – Evans, H.F., Harrap, K.A. (1982), Persistence of Insect Viruses, In: Mahy, B.W.J., Minson, A.C., and Darby, G.K. (eds) Virus persistence: Thirty-third Symposium of the Society for General Microbiology held at the University of Cambridge, March 1982, pp. 57-96, Cambridge University Press, Cambridge
Published report
BVL no 3682785

Abstract: One of the features which influences the interaction of insects and their viruses is the discontinuity of the host population. Most insects have a dormant phase where metabolic activity is extremely low and this stage is frequently unavailable for virus infection. In such circumstances the virus has to persist in an infective state until the host is once more available for infection. Persistence of insect viruses therefore means their survival in the natural environment as in most instances insect viruses do not require intimate host-to-host transfer to retain viability. The means by which insect viruses can persist in nature are important both in the initiation and maintenance of infection in a population. The two key features of this persistence are the preservation of virus infectivity and the ways in which transmission to a susceptible host can occur. These are influenced by many factors and their interrelationship is examined in this chapter.

Previous evaluation: **in DAR (2007)**
Evaluation by the RMS (2019): **Relevant and reliable but due to publication date possibly not reflecting the current state of knowledge.**

Report KMA 2.4 – OECD (2002), Consensus Document on Information used in the Assessment of Environmental Applications involving *Baculovirus* (ENV/JM/MONO(2002)1), Series on Harmonization of Regulatory Oversight in Biotechnology, No.20, OECD Environment Directorate, Paris. (Available on the Biotrack website at http://www.oecd.org/biotrack/)
Published report
BVL no 2019094

Abstract: The OECD's Working Group on Harmonization of Regulatory Oversight in Biotechnology decided at its first session, in June 1995, to focus its work on the development of consensus documents which are mutually acceptable among Member countries. These consensus documents contain information for use during the regulatory assessment of a particular product. This document contains general information on baculoviruses such as organism characteristics, behavior in the environment, their history of use and interactions, as well as environmental safety considerations. Germany served as lead country in the preparation of this document. It has been revised on a number of occasions based on the input from other Member countries. It is intended for use by regulatory authorities and others who have responsibility for assessments and by those who are actively involved with genetic improvement and intensive management of the genus.

Previous evaluation: **in DAR (2007)**
Evaluation by the RMS (2019): **Relevant and reliable.**

Report KMA 2.4 – Copping, L.G., (1998). 1:36 *Cydia pomonella* granulosis virus. In: Copping, L.G. (ed.) (1998), The BioPesticide Manual. A world compendium, 1st edition, British Crop Protecion Council, pp. 60-61, Farnham, UK
Published report
BVL no 3682777

Abstract: The nomenclature of *Cydia pomonella* granulosis virus, its source, production, target pests and target crops, its biological activity, commercialization, application, product specifications, and compatibility, as well as its mammalian toxicity, environmental impact and non-target toxicity are shortly summarized. The nomenclature of *Cydia pomonella* granulosis virus, its source, production,

target pests and target crops, its biological activity, commercialization, application, product specifications, and compatibility, as well as its mammalian toxicity, environmental impact and non-target toxicity are shortly summarized.

Previous evaluation:	**in DAR (2007)**
Evaluation by the RMS (2019):	**Relevant for the DAR but possibly not reflecting the current state of knowledge. This manual has several times been revised. The most recent edition (fifth edition) has been published in 2014. Furthermore, an online version containing up-to-date information has been made available by the publisher (see links provided below).** https://www.bcpc.org/product/manual-of-biocontrol-agents-fifth-edition https://www.bcpc.org/product/manual-of-biocontrol-agents-online

Report KMA 2.4 – Hess, R.T., Falcon, L.A. (1987). Temporal Events in the Invasion of the Codling Moth, *Cydia pomonella*, by a Granulosis Virus: An Electron Microscope Study, Journal of Invertebrate Pathology, 50, 85-105
Published report
https://doi.org/10.1016/0022-2011(87)90108-X
BVL no 2019102

Abstract: The replication cycle of the granulosis virus of *Cydia pomonella*, the codling moth, was studied at the cellular and tissue level. Membranelike complexes were observed forming within the remnants of the nucleolus in the cytoplasm of infected cells. Differences in cell polarity relative to the sites of virus entry assembly and budding as well as differences in the temporal aspects of replication were observed between midgut, fat body, and epidermal cells. The progressive spread of virus throughout larval tissues was studied at 24, 32, 48, 56, and 72 hr postinfection. The basal lamina seemed to be an effective barrier for the release of budded progeny virus into the hemocoel and large numbers of budded virus were produced.

Previous evaluation:	**in DAR (2007)**
Evaluation by the RMS (2019):	**Relevant and reliable.**

Report KMA 2.4 – Jaques, R.A. (1977), Stability of Entomopathogenic Viruses, In: Ignoffo, C.M., and Hostetter, D.L. (eds), Miscellaneous Publications of the Entomological Society of America, pp. 99-116
Published report
BVL no 3728861

Abstract: The nuclear-polyhedrosis and granulosis viruses, the types of insect viruses being considered for development as microbial insecticides, are quite stable at moderate and low temperatures, may remain active for long periods in soil, are not affected directly by humidity, and are compatible with the majority of chemical insecticides. These viruses are readily inactivated by exposure to sunlight, strong acids or alkalis, and high temperatures. This discussion indicates that inactivation by sunlight is the most important factor causing loss of activity of viruses in the field environment.

Previous evaluation:	**in DAR (2007)**
Evaluation by the RMS (2019):	**Relevant and reliable.**

Report KMA 2.4 – Bilimoria, S.L. (1986), Taxonomy and Identification of Baculoviruses, In: Granados, R.R and Federici, B.A. (eds.), The Biology of Baculoviruses, Volume 1: Biological Properties and Molecular Biology, pp. 37-59. CRC Press, Boca Raton
Published report
BVL no 3689461

Abstract: This chapter describes the basic properties of the major subgroups of baculoviruses and reviews the most useful and practical approaches for their identification and classification. Special emphasis is placed on the impact of recent technological developments on baculovirus taxonomy. Several previous reviews have addressed the question of the classification and identification of baculoviruses, and the reader may wish to consult these for earlier developments about which detailed information may not be provided in the following account. Longworth recently discussed current problems in insect virus taxonomy.

Previous evaluation:	**in DAR (2007)**
Evaluation by the RMS (2019):	**Possibly relevant, however, only in the context being used. Otherwise this review is really dated, especially with reference to taxonomy.** **Regarding taxonomy the right source would be: Herniou, E. A., Arif, B. M., Becnel, J. J., Blissard, G. W., Bonning, B., Harrison, R., Jehle, J. A., Theilmann, D. A. and Vlak, J. M., Baculoviridae. In: King, A. M. Q., Adams, M. J., Carstens, E. B., Lefkowitz, E. J. (editors). *Virus Taxonomy*. Oxford: Elsevier; 2011. pp. 163–174.** **Regarding the life cycle of baculoviruses the more appropriate source would perhaps have been: Slack and Arif, 2006, The Baculoviruses Occlusion-Derived Virus: Virion Structure and Function, Advances in Virus Research 69, 99-165, http://dx.doi.org/10.1016/S0065-3527(06)69003-9**

Report KMA 2.4 – Kool, M., Ahrens, C.H., Vlak, J.M., Rohrmann, G.F. (1995). Replication of Baculovirus DNA, Journal of General Virology, 76, 2103-2118
Published report
https://doi.org/10.1099/0022-1317-76-9-2103
BVL no 2019110

Abstract: The Baculoviridae is a diverse family of pathogens that are infectious for arthropods and are characterized by a complex replication cycle that culminates in the occlusion of virions in a crystalline protein matrix. Over 400 lepidopteran species serve as hosts for these viruses, with a single virus isolate usually restricted to one or a few related species. In addition, they have been reported from species of the orders Hymenoptera, Diptera, Siphonoptera and Trichoptera, as well as in several crustaceans. Baculoviruses are divided into two genera based on occlusion body morphology; the nuclear polyhedrosis viruses (NPVs), which are characterized by many virions present in each polyhedron-shaped occlusion body, and the granulosis viruses (GVs), which normally have smaller occlusion bodies each containing only a single virion, and have been reported to infect only the Lepidoptera. Occlusion bodies function to protect virions against proteolysis and environmental decay; occluded virions may remain viable for many years in the environment.

Previous evaluation:	**in DAR (2007)**
Evaluation by the RMS (2019):	**Supplementary information. Not necessarily relevant unless molecular events are generally included in the description of the virus's life cycle.**

Report KMA 2.4/01 – Huh, N. E., Weaver, R. F. (1990). Identifying the RNA polymerases that synthesize specific transcripts of the *Autographa californica* nuclear polyhedrosis virus, Journal of General Virology, 71, 195-201 Published report https://doi.org/10.1099/0022-1317-71-1-195 BVL no 3714815
Abstract: Nuclear run-on assays carried out in the presence and absence of the RNA polymerase II inhibitor, α-amanitin, were used to determine the exact timing of the switch from inhibitor-sensitive transcription catalysed by host RNA polymerase II, to inhibitor-resistant transcription catalysed by the baculovirus-induced RNA polymerase. These studies revealed that the onset of α-amanitin-resistant transcription is just after 6 h post-infection, simultaneous with the beginning of the late phase of infection. They also showed that transcripts from the p26 gene in the *Hin*dIII Q/P region and the p35 gene in the *Hin*dIII K/Q region of the viral genome are synthesized by the host RNA polymerase II both early and late in infection. On the other hand, transcripts of the p10 gene in the *Hin*dIII Q/P region and the γ transcripts in the *Hin*dIII K region are synthesized by the α-amanitin-resistant, virus-induced RNA polymerase late in infection.
Evaluation by the RMS (2019): Submitted for the purpose of renewal **Supplementary information. Not necessarily relevant unless molecular events are generally included in the description of the virus's life cycle.**

B.2.5 Infectiveness, dispersal and colonisation ability

The following information, highlighted in grey, is derived from the Draft Assessment Report Volume 3, Annex B-2, Point B.2.1.5.

A summary on the environmental stability of baculoviruses has been issued by Jaques (1977, BVL no 3714768), which includes the results of all older laboratory and field studies of importance. The summary of Jaques has been further compressed and together with supplementary results of newer studies, forms the base of this compilation. In the following chapters all references marked with * are cited by Jaques (1977).

B.2.5.1 Effects of sunlight

Sunlight is considered the most important factor contributing to the inactivation of viral occlusion bodies. Jaques (1972*) showed that *Trichoplusia ni* NPV and *Pieris rapae* GV applied to leaves of cabbage in field plots lost at least 50% of original activity within 2 days and retained less than 15% of original activity after 10 days (see Figure B.2.5-1).

Figure B.2.5-1: Activity of *Trichoplusia ni* NPV and *Pieris rapae* GV following application of suspensions of occlusion bodies to leaves of cabbage in field plots

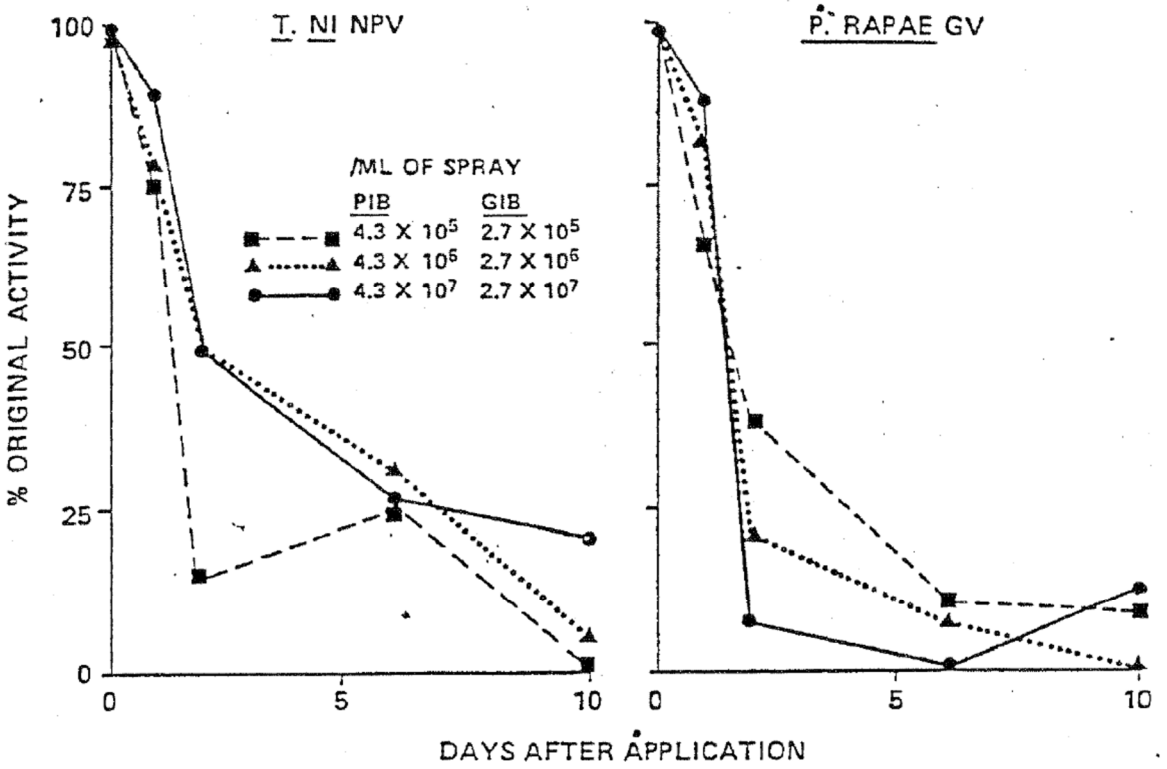

Fig. 1.--Activity of *Trichoplusia ni* NPV and *Pieris rapae* GV following application of suspensions of inclusion bodies to leaves of cabbage in field plots (from Jaques 1975).

Similar results of inactivation were obtained with:

- *Heliothis* NPV on cotton (Bullock, 1967*; Ignoffo and Batzer, 1971*), corn silks (Ignoffo *et al.*, 1973*), soybean foliage (Ignoffo *et al.*, 1974*), and on glass plates (Ignoffo and Batzer, 1971*)
- *Epiphyas postvittana* NPV on apple leaves (Mac Collom and Reed, 1971*)
- *Cydia pomonella* GV and *Adoxophyes orana* NPV on apple leaves (Huber, 1982, BVL no 3682714)
- *Pieris brassicae* GV on cabbage (David *et al.*, 1968*)
- *Zeiraphara diniana* GV on larches (Schmid, 1974, BVL no 3682823)
- *Lambdina fiscellaria somniaria* NPV suspensions exposed to sunlight (Morris, 1971*)
- *Trichoplusia ni* NPV on Millipore filters exposed to sunlight (Cantwell, 1967*)

Steineke (2004, BVL no 2019090) infected apples with CpGV and exposed them to sunlight for various times. The activity of CpGV after single application was measured in a treated and an untreated group (each 50 apples). Treated apples caused an exponentially decreasing mortality among larvae which were applied after harvesting the apples. Mortality dropped from around 80% to about 1% after 300 hours of sunlight (more than 12 days). The calculated half-life was around 52.2 sunlight hours.

Deposits of *T. ni* NPV and *P. rapae* GV on plants in a dark cupboard lost little activity in the 20-day test, indicating that exposure to light, especially sunlight, was the major factor contributing to inactivation (Jaques, 1967a*, 1972a*).

There is considerable evidence that it is the ultraviolet portion of sunlight that inactivates insect viruses.

Krieg *et al.* (1981, BVL no 3682824) determined the sensitivity of five insect pathogens to short-wave UV light (254 nm) and to longer-wave UV light (285-380 nm). The results are summarised in Table B.2.5-1 and Table B.2.5-2Table B.2.5-2.

Table B.2.5-1: Comparison of the stability of certain insect pathogens after irradiation with a "Sterisol" lamp (far UV: 254 nm; illumination rate 0.046 mW/cm²)

Pathogen (irradiated object)	Inactivation rate (%)	Inactivation dose (mW sec/cm²)
Bacillus thuringiensis (vegetative cells)	90 99 99.9	2.6 5.0 7.5
Bacillus thuringiensis (spores)	90 99 99.9	6.7 13.6 20.4
Beauveria bassiana (conidia)	50 90 99	17.1 34.5 43.6
Mamestra brassicae Nucleopolyhedrovirus (polyhedra)	90 99	11.9 46.9
Laspeyresia (= *Cydia*) *pomonella* Granulovirus (granules)	99 99.9	32.0 195.6

Table B.2.5-2: Comparison of the stability of certain insect pathogens after irradiation with "Ultra-Vitalux" lamps (near UV: 285-380 nm; illumination rate 0.5 mW/cm² at 285-315 nm and 2.5 mW/cm² at 315-380 nm)

Pathogen (irradiated object)	Inactivation rate (%)	Inactivation dose (mW sec/cm²)
Bacillus thuringiensis (vegetative cells)	90 99 99.9	41 + 213 81 + 421 120 + 624
Bacillus thuringiensis (spores)	90 99 99.9	104 + 541 214 + 1113 318 + 1654
Beauveria bassiana (conidia)	50 90 99	531 + 2761 774 + 4024 942 + 4898
Mamestra brassicae Nucleopolyhedrovirus (polyhedra)	90 99	321 + 1669 918 + 4774
Laspeyresia (= *Cydia*) *pomonella* Granulovirus (granules)	99 99.9	189 + 983 615 + 3198

Short-wave UV light had a considerably higher germicidal effect for all pathogens tested than long-wave UV. Rapid inactivation at short-wave UV light (254 nm) was also observed with:

- - *Heliothis* NPV (Gudauskos and Cannerday, 1968*; Ignoffo and Batzer, 1971*)
- - *Trichoplusia ni* NPV (Jaques, 1967a*, 1968*, 1971*; Gudauskos and Cannerday, 1968*)

- - *Zeiraphera diniana* GV (Schmid, 1974)

Bullock *et al.* (1970*) found that exposure of suspensions of *Heliothis* NPV to UV light (254 nm) reduced activity more than to UV light (307.5 nm) while exposure to longer-wave UV (364 nm) and a broad-band mixture of visible and IR light did not affect activity. Likewise David (1969*) noted that the effect of UV light on *P. brassicae* GV decreased as the wavelength was increased from 250 nm to 320 nm. Exposure to high dosages of longer wavelengths had no detectable effect on this virus. Morris (1971*) exposed *Lambdina fiscellaria lugubrosa* NPV to UV light (366 nm) for long periods without causing appreciable effect on the virus. Smirnoff (1972*) showed that the intensity of the UV portion of sunlight as well as its wavelength affected the rate of inactivation of *Neodiprion swainei* NPV.

The rapid inactivation of foliar deposits of viruses by sunlight and UV light led to a search for materials that could protect viral sprays to prolong their activity and increase their effectiveness in control. Whereas, in laboratory experiments, deposits of *T. ni* NPV applied alone were inactivated in 5 days, deposits of this virus applied with a brewers yeast-charcoal or a skim milk-charcoal mixture retained over 80% of original activity 15 days after application. Similar results were obtained with *P. rapae* GV (Jaques 1971*, 1972a*). Different light absorbing substances, i.e. 2% dried skimmed milk powder or 1% milk + 1% Indian ink which were added to suspensions of *Zeiraphera diniana* GV slowed down the inactivation process considerably. Similar results were obtained with 2% sucrose, but the mechanism of its protective action is not well understood (Schmid, 1974). Also field tests revealed promising results by mixtures of carbon with *Heliothis* NPV (Ignoffo and Batzer, 1971*) and addition of skim milk-charcoal or egg albumin-charcoal to *T. ni* NPV or *P. rapae* GV (Jaques 1971*, 1972a*).

Forms of irradiation other than UV and a part of the visible spectrum appear to have little effect on insect viruses. This was observed at exposure of dried deposits of *P. brassicae* GV to infrared light (David *et al.*, 1971a*). Similarly, gamma radiation had only a moderate effect on activity of *T. ni* NPV (Jaques, 1968*) and of *L. f. somniara* NPV (Morris, 1971*).

It is well known that most of the CpGV applied with a treatment is inactivated rather quickly by UV-irradiation with a half life of about two days (Kienzle *et al.*, 2003 and references therein, BVL no 3682888). Thus, frequent treatments are believed to be inevitable for CpGV. However, at least two studies showed, that a small part of the CpGV persists for much longer time in the orchard (Kienzle *et al.*, 2003 and references therein). Laboratory findings indicate that the UV-inactivation of CpGV curve is bi-shaped. This means, that most of the CpGV (about 99%) is inactivated very fast, a small part, however, is subjected to a much lower inactivation (Kienzle *et al.*, 2003 and references therein).

B.2.5.2 Effects of temperature

Virus suspensions or dried powders remain active for long periods if they are kept at low temperatures (Table B.2.5-3).

Table B.2.5-3: The effect of storage at low temperature on viral activity

Virus	Storage temperature (°C)	Period of storage (years)	Loss of activity (Estimated %)	Reference
Bombyx mori NPV (Ampules)	4	20	<50	Steinhaus* 1960
Neodiprion hercyniae NPV (Cadavers)	4	6	<25	Neilson and Elgee* 1960
Lambdina f. fiscellaria NPV (Suspension)	4	6	>90	Cunningham* 1970
Trichoplusia ni NPV (Suspension)	4	4	<10	Jaques* (Unpublished)
Pieris brassicae GV (Dry)	0	4	<10	David and Gardiner 1967

Virus	Storage temperature (°C)	Period of storage (years)	Loss of activity (Estimated %)	Reference
Pieris rapae GV (Suspension)	4	4	<10	Jaques* (Unpublished)
Panonychus citri virus (Dry)	4	6.5	<10	Shaw* et al. 1972
Cydia pomonella GV (suspension)	5-8	>2	<10	Huber (2000)

David *et al.* (1971a*) froze and thawed suspension of *P. brassicae* GV 10 times in 12 days without causing a significant loss of activity indicating that repeated freezing and thawing of a virus in the field environment would not affect activity appreciably.

Exposure to high temperatures causes inactivation. Results of several studies show that a 10-minute exposure to temperatures of 70-80°C would be expected to inactivate an insect virus. If the CpGV formulation Granupom is stored at temperatures above 54°C for more than 14 days it becomes biologically unstable and loses its efficacy (Gröner *et al.*, 1990, BVL no 3682712). Because temperatures in the field do not reach these high levels, studies of long exposures to moderately high temperatures are of more practical interest in considering stability of microbial insecticides in the field environment. Hunter *et al.* (1973*) showed that *Cadra cautella* NPV in bran diet lost little activity in 28 days at 32°C, but was 80% inactivated at 42°C for the same period. The virus withstood 7 days exposure to 42°C and was only partially inactivated in 14 days at this temperature. Similarly David and Gardiner (1967b*) showed that dried films of *P. brassicae* GV were not completely inactivated at 40°C in 20 days. These studies indicate that the viruses should withstand higher temperatures in the field, at least for short periods.

B.2.5.3 Effects of humidity

It is generally accepted that humidity has less effect on stability of insect viruses than on stability of other types of pathogens (Table B.2.5-4).

Table B.2.5-4: The effect of rainfall and high humidity on the persistence of insect viruses

Virus	Treatment	Result	Author(s)
Thaumetopoea pityocampa LPV on pine foliage	50 mm simulated rain	little loss in activity	Bergerjon and Grison, 1965*
Zeiraphera diniana GV on larch foliage	comparison at cool rainy weather and fine dry weather	the proportion in activity loss remained the same	Schmid, 1974
Heliothis NPV on cotton	irrigation	no loss in activity	Bullock, 1967* Ignoffo et al., 1965*
P. brassicae GV on cabbage leaves	a) pure preparation b) crude preparation 250 cm simulated rain for 5 h	no loss in activity	David and Gardiner, 1966*
P. brassicae GV on glass slides	exposure to high humidity at 20°C for 7 days compared to low humidity	inactivation was slightly greater at low humidity	David et al., 1971b*
T. ni NPV on cabbage leaves	leaves placed for 4 days in a photoprint washer	only 50% loss in activity	Jaques, 1967a*
T. ni NPV on cabbage leaves	plants placed under a lawn sprinkler during sunshine	only slightly more loss than at dry plants	Jaques, 1967a*

Virus	Treatment	Result	Author(s)
T. ni NPV, *P. brassicae* GV on cabbage leaves	wetted deposits compared with dry ones, exposure to UV light	wetted deposits were inactivated more quickly than the dry ones	Jaques, 1967a* David, 1969*

The trials indicate that humidity does not show direct influence on viral stability. However, there may be an indirect influence by affecting chemical action on the virus and by increasing the inactivation rate by sunlight.

B.2.5.4 Effects of substrate:

Foliage, fruits:
The inactivation of virus by exposure to sunlight is the most important factor. Naturally, virus deposits on leaves/fruits on the inside of the foliage canopy are more protected than those of the periphery. Certain substances have negative impacts on the stability of viruses. Leaf exudates may produce alkaline layers of pH up to 10.1 (Andrews and Sikorowski, 1973*) and high concentrations of metallic ions (McLeod *et al.*, 1977; cited by Evans and Harrap, 1982, BVL no 3714767). However, these observations were done on *Heliothis* NPV and do not exist for CpGV on fruit trees. Nevertheless, attention must be drawn to the fact that tank-mixtures or sequences of sprays may inactivate CpGV if they form alkaline and/or metallic ions containing deposits.

Soil:
Viruses may persist in soil for longer periods (see Table B.2.5-5).

Table B.2.5-5: Persistence of insect viruses in soil

Virus	Duration in soil	Results	Author(s)
T. ni NPV	6 years; application to surface in field plots	still about 15 % of its original activity retained	Jaques, 1964*
T. ni NPV	1 year	accumulation in soil	Thomas et al., 1972*
T. ni NPV *P. rapae* GV	1 foliar application to cabbage plots, 5 year larval biotest of soil	accumulation during the 1st year after application, then same level as in control plots	Jaques, 1974a* Jaques, 1974b*
P. brassicae GV	2 years in soil or sand	lost little activity only	David and Gardiner, 1967
Hyphantria lunea NPV	8 month in soil at room temperature	lost little activity only	Hukahara and Namura, 1972*
Several viruses	Californian field	Authors concluded that the viruses had accumulated from epizootics of the diseases	Tanada and Omi, 1974

The pH of the soil may affect persistence of viruses. The adverse affect of extreme hydrogen ion concentration buffers on the infectivity of NPVs has been reported by Ignoffo and Garcia (1966*) and Gudauskas and Cannerday* (1968). Thomas *et al.* (1973, BVL no 3682710) bioassayed *T. ni* NPV extracts of a loamy sand of various pH at three-monthly intervals and showed a correlation between pH and virus activity: the lower the pH, the more rapidly the virus was inactivated (see Table B.2.5-6).

Table B.2.5-6: Average activity of *Trichoplusia ni* polyhedral occlusion bodies (PIBs) recovered from 3 replicates of pH adjusted soils sprayed with 100 larval equivalents per acre

	Avg. No. PIBs/500 g soil sample x 10^4 recovered at 3 monthly intervals after treatment			
Avg. pH	3	6	9	12
4.83	40.5	20.6	9.3	-
5.02	44.3	8.3	5.0	-
5.22	70.4	21.1	4.1	-
6.05	186.6	55.3	62.1	24
7.00	320.1	192.4	127.5	106.6
7.17	401.0	277.8	193.9	79.0
[a]no. of PIBs recovered =	$\frac{\text{Virus Std. } LD_{50} \text{ (PIB/g diet) x Wt. of soil sample (g) x Wt. of bioassay diet (400 g)}}{LD_{50} \text{ of soil sample (g soil)}}$			

Water:
While it is known that most viruses in intact occlusion bodies are reasonable stable in aqueous suspension, little is known of their persistence in natural aquatic environment. It is supposed that the pH and salt concentration of water would influence stability.

B.2.5.5 Dispersal routes

Dispersal of baculoviruses in general includes small animals and birds (their faeces are able to contain infective viruses), predators, wind blow of dry soil and rain splash at canopy edges. Knowledge of the importance of such mechanisms is scant (Evans and Harrap, 1982).

New data 2016
No new data have been submitted under this point. The notifier considers the previously submitted information to be acceptable to cover current requirements. No additional references were identified by the notifier from peer-reviewed open literature to be relevant in regard to the data point "Infectiveness, dispersal and colonisation ability". Please refer to the "References relied on" section (chapter B.2.10) for a summary and an evaluation of the literature search.

B.2.5.6 Conclusion by the RMS (2019)

According to Commission Regulation (EU) No 283/2013 (Annex Part B Micro-organisms including viruses) five points need to be addressed in chapter B.2.5. These data requirements are outlined below followed by a short evaluation whether or not they have been adequately addressed by the notifier.

(i) "The persistence of the micro-organism and information on its life cycle under the typical environmental conditions of use must be indicated. In addition, any particular sensitivity of the micro-organism to certain compartments of the environment (e.g. UV light, soil, water) must be stated."

While much information has been provided on the persistence of nucleopolyhedroviruses and granuloviruses in regard to UV-light in general only little information has been given concerning the virus of interest, i.e. CpGV. In fact, only three publications have been cited that deal with the persistence of CpGV when exposed to sunlight, i.e. Krieg *et al.* (1981, BVL no 3682824), Huber (1982, BVL no 3682714), and Steineke (2004, BVL no 2019090). Although the information that sunlight is considered the most important factor contributing to the inactivation of viral occlusion bodies is definitely relevant there is a great distortion towards baculoviruses other than CpGV. The

two only specific information given for CpGV are (i) its half-life under sunlight and (ii) the inactivation doses needed to inactivate 99% and 99.9% of CpGV in the far UV (254 nm) and near UV (285-380 nm).

As regards the sensitivity of CpGV to different environmental compartments the same holds true: much information in general on the persistence of nucleopolyhedroviruses and granuloviruses on foliage, fruits, soil, and water but no specific data concerning the sensitivity of CpGV in these compartments.

Notably, no literature search was performed.

(ii) "The environmental requirements (temperature, pH, humidity, nutrition requirements, etc.) for survival, reproduction, colonisation, damage (including human tissues) and effectiveness of the micro-organism must be stated. The presence of specific virulence factors shall be indicated."

Similar to what is stated above some specific information has been buried under a heap of general information. After all, only two publications deal with the environmental requirements of CpGV, i.e. Huber (2000, BVL no 3682728) and Gröner *et al.* (1990, BVL no 3682712). These publications showed that CpGV can be stored for two years at 5-8°C without losing any activity and that it loses its efficacy if stored at temperatures above 54°C for more than 14 days, indicating that CpGV will likely also persist in the environment at lower temperatures but not at higher temperatures.

As regards pH and humidity no specific information has been provided for CpGV. However, the general information given indicate that an acidic pH has a negative effect on the persistence/ survival of the virus and that humidity does not show direct influence on viral stability.

Again and as before, no literature search was performed.

(iii) "The temperature range at which the micro-organism grows must be determined, including minimum, maximum and optimum temperatures. This information is of particular value as a trigger for studies of effects on human health."

No information has been provided in regard to this data point and no literature search was performed.

(iv) "The possible effect of factors such as temperature, UV light, pH, and the presence of certain substances on the stability of relevant toxins must also be stated."

As stated by the notifier in the introductory part no metabolites are produced by CpGV. Therefore, this data point does supposedly not apply. However, the notifier's introductory statement has not been backed up by recent references. Moreover, neither "metabolite" nor "toxin" were included as specific search terms in the search strategy. Thus, a literature search covering the last ten years would be necessary to maintain the previous evidence that no metabolites and toxins are produced by CpGV.

(v) "Information on possible dispersal routes of the micro-organism (via air as dust particles or aerosols, with host organisms as vectors, etc.), under typical environmental conditions relevant to the use, must be provided."

Little information has been provided regarding this data requirement. The notifier states that knowledge concerning dispersal routes is scant and refers to a review published in 1982. Obviously, this reference cannot be considered current state of knowledge.

Finally, no literature search was performed on this point either.

The notifiers conclusion that no additional references were identified from peer-reviewed open literature to be relevant for the chapter on infectiveness, dispersal and colonisation ability is evident as the search strategy did not include any of the specific search terms relevant for this chapter, e.g.: (i) “persistence” OR “sensitivity” AND (“UV” OR “light” OR “soil” OR “water”), (ii) “survival” OR “reproduction” OR “colonization” OR “effectiveness” AND (“temperature” OR “pH” OR “humidity”), (iii) “virulence”, (iv) “metabolite” OR “toxin”, (v) “dispersal”, or other search terms that could in any way be appropriate.

B.2.5.7 Cited references

Report KMA 2.5 – Jaques, R.A. (1977), Stability of Entomopathogenic Viruses, In: Ignoffo, C.M., and Hostetter, D.L. (eds), Miscellaneous Publications of the Entomological Society of America, pp. 99-116
Published report
BVL no 3714768

Abstract: The nuclear-polyhedrosis and granulosis viruses, the types of insect viruses being considered for development as microbial insecticides, are quite stable at moderate and low temperatures, may remain active for long periods in soil, are not affected directly by humidity, and are compatible with the majority of chemical insecticides. These viruses are readily inactivated by exposure to sunlight, strong acids or alkalis, and high temperatures. This discussion indicates that inactivation by sunlight is the most important factor causing loss of activity of viruses in the field environment.

Previous evaluation:	**in DAR (2007)**
Evaluation by the RMS (2019):	**Relevant in regard to nucleopolyhedroviruses and granuloviruses in general but irrelevant in regard to CpGV in particular. Furthermore, due to publication date possibly not reflecting the current state of knowledge.**

Report KMA 2.5 – Huber, J. (1982), Comparison of Field Persistence of CpGV and AoNPV, In: C.E.C. meeting of experts on "Virus production and Specific Control Techniques in Orchards", Darmstadt, 2-3 December, 1982
Published report
BVL no 3682714

Abstract: In the last ten years a lot of information has been published on the UV-sensitivity of different insect viruses. Halflife values ranging from a few hours up to several days have been reported. Unfortunately these data are hardly comparable: The activity of a virus preparation can only be determined in a bioassay, using the host insect as test animal. Since insect viruses are rather specific, for most of them different test insects and bioassay systems have to be used. Probably, the wide differences in UV-stability data in literature do not reflect real differences in sensitivity of the viruses, but are merely an artifact created by the different bioassay techniques used. The aim of this study was therefore to compare the field persistence of a GV from the codling moth, *Cydia pomonella*, and of a NPV from the summer fruit tortrix, *Adoxophyes orana*, under most identical conditions.

Previous evaluation:	**in DAR (2007)**
Evaluation by the RMS (2019):	**Relevant and reliable.**

Report KMA 2.5 – Schmid, A. (1974). Investigations of the Persistance of the Granulosis Virus of the Larch Bud Moth *Zeiraphera diniana* (Gn.) in the Environment and the Protective Action of Some Substances, Zeitschrift für Angewandte Entomologie, 76, 31-49
Published report
https://doi.org/10.1111/j.1439-0418.1974.tb01866.x
BVL no 3682823

Abstract: Investigations on the persistency of virus spray covers on larch trees in the Engadin (1800 m above sea level) and in Zurich (600 m) showed that the virus is quickly inactivated by sunlight and especially light of short wave lengths. Different light absorbing substances, i.e. 2% dried skimmed milk powder or 1% milk + 1% Indian ink, which were added to the virus suspensions, slowed down the inactivation process considerably. Similar results were obtained with 2% sucrose, but the mechanism of its protective action is not well understood. Buffering of virus suspensions and rain have little effect. The same is true concerning secondary plant substances that might be emanated by the larch tree. At 1800 m above sea level inactivation of virus sprays, even when protected by the above mentioned substances, proceeds so quickly that there is little chance that the virus persists on the trees from one year to the next to such an extent that it might declenche an epizootic. Two virus suspensions with and without phosphate buffer had the same infective potency after 15 months storage at 2°C. A freeze dried sample of a virus suspension containing 5% cellulose powder was 3.5 times more infective after 8 months at 2°C than a sample of the same suspension stored at the same temperature in the liquid form.

Previous evaluation: **in DAR (2007)**
Evaluation by the RMS (2019): **Supplementary information.**

Report KMA 2.5 – Steineke, S.B. (2004), Populationsdynamik des *Cydia pomonella* Granulovirus, Dissertation zur Erlangung des Grades "Doktor der Naturwissenschaften" am Fachbereich Biologie der Johannes Gutenberg-Universität in Mainz, pp. 134
Published report
BVL no 2019090

Abstract: The *Cydia pomonella* Granulovirus (CpGV, Fam. Baculoviridae), an extremely virulent and highly specific pathogen, has been registered for the control of the codling moth (*Cydia pomonella*) in Germany and other countries of the EU. It infects the larval stages of its host and does not harm non target organisms. Past research on CpGV addressed questions relevant to its production and application as pest control agent. However, 20 years after the first registration, it remains unclear whether CpGV can establish itself in the environment. As part of this project, various parameters were analysed and quantified to aid in describing CpGV's population dynamics. The studied parameters included virulence, virus yield, horizontal and vertical transmission, inactivation rate and the infection rate of late instars. The quantified parameters were then integrated into a mathematical model along with data found in the relevant literature.

For the extended abstract please refer to:
https://publications.ub.uni-mainz.de/theses/frontdoor.php?source_opus=659

Previous evaluation: **in DAR (2007)**
Evaluation by the RMS (2019): **Relevant and reliable.**

Report KMA 2.5 – Krieg, A., Gröner, A., Huber, J., Zimmermann, G. (1981). Inactivation of Certain Insect Pathogens by Ultraviolet Radiation, Journal of Plant Diseases and Protection, 88 (1), 38-48
Published report
http://www.jstor.org/stable/43214776
BVL no 3682824

Abstract: The UV-sensitivity of two baculoviruses (granulosis virus, nuclear polyhedrosis virus) and two entomopathogenic microorganisms (*Bacillus thuringiensis*, *Beauveria bassiana*) was determined by radiation tests. In the far UV (254 nm) the stability, measured at an inactivation rate of 99%, was in declining order: nuclear polyhedra ≥ conidia of *B. bassiana* > granula > spores of *B. thuringiensis* ≥ vegetative cells of *B. thuringiensis*. In the near UV (285-380 nm) the following order could be found: conidia of *B. bassiana* ≥ nuclear polyhedra > spores of *B. thuringiensis* ≥ granula > vegetative cells of *B. thuringiensis*. Far UV had a much higher germicidal effect for all pathogens tested than near UV.

Previous evaluation: **in DAR (2007)**
Evaluation by the RMS (2019): **Relevant and reliable.**

Report KMA 2.5 – Kienzle, J., Schulz, C, Zebitz, C.P.W., Huber, J. (2003), Persistence of the Biological Effect of Codling Moth Granulovirus in the Orchard - Preliminary Field Trials, In: Papierok, B. (ed.) IOBC/WPRS Bulletin Vol. 26 (1), Working Group "Insect Pathogens and Insect Parasitic Nematodes", Proceedings of the 8th European Meeting "Entomopathogens and Insect Parasitic Nematodes: Current Research and Perspectives in Pest Biocontrol" at Athens (Greece), 29 May - 2 June 2001, pp. 245-248.
Published report
BVL no 3682888

Abstract: In 2000 and 2001, in a field trial, the persistence of the biological effect of codling moth granulovirus (CpGV) was investigated. With a single treatment at full concentration of CpGV (MADEX 3, 100 ml/ha) a considerable reduction of CM population was achieved over the whole vegetation period. This may indicate, that over a considerable period of time after a treatment a biological effect of CpGV sufficient for an increased mortality of the larvae was present in the orchard. However, the onset of mortality was not fast enough to protect the fruit from damage. Further research has to be done to gain more experience in handling this effect. It could be very important for the reduction of the number of treatments in organic apple growing.

Previous evaluation: **in DAR (2007)**
Evaluation by the RMS (2019): **Relevant and reliable, however, it should be noted that the publication copy provided is not the one that has been cited. The copy provided (which is similar in content to the cited publication) has already been published in 2002 and comes from a different source, i.e.:**

Kienzle, J. Schulz, C., Zebitz, C.P.W., Huber, J. (2002). Persistence of the biological effect of codling moth granulovirus in the orchard - preliminary field trials, 10th International Conference on Cultivation Technique and Phytopathological Problems in Organic-Fruit-Growing and Viticulture, Proceedings to the conference from 4th to7th February 2002 at Staatliche Lehr- und Versuchsanstalt für Wein- und Obstbau Weinsberg, pp. 187-191

Report KMA 2.5 – David, W.A.L., Gardiner, B.O.C. (1967). The Persistence of a Granulosis Virus of *Pieris brassicae* in Soil and in Sand, Journal of Invertebrate Pathology 9, 342-347
Published report
https://doi.org/10.1016/0022-2011(67)90068-7
BVL no 3682713

Abstract: A granulosis virus from *Pieris brassicae* larvae was found to be very stable in garden soil and sand and showed little deterioration after 2 years. The virus could not be readily washed out of the soil or sand. When water equivalent up to 48 inches of rain was passed through the samples much of the virus remained in the top layer though some was carried away in the percolating water.

Previous evaluation: **in DAR (2007)**
Evaluation by the RMS (2019): **Supplementary information.**

Report KMA 2.5 – Huber, J. (2000). Certificate of Analysis, Determination of the Activity of Codling Moth Granulosis Virus in a Sample Received from PROBIS, Pforzheim (Shelf-life study)
unpublished
BVL no 3682728

Abstract: Assay method: Bioassays following the diet incorporation method by Huber (Mitt-Deutsch. Ges. Allg. Angew. Entomol. 2, 141-145, 1981) using neonate larvae of codling moth on artificial diet. For each assay 5 concentrations à 50 larvae were used. Conclusions: During more than 2 years of storage the virus preparation did not lose any activity.

Previous evaluation: **in DAR (2007)**
Evaluation by the RMS (2019): **Relevant and reliable.**

Report KMA 2.5 – Gröner, A., Knauf, Reuß (1990). *Cydia pomonella* Granulosus Virus (CpGV) Hoe 083311 – Summary on Chemical and Physical Data
unpublished
BVL no 3682712

Abstract: Study report without abstract and/or summary.

Previous evaluation: **in DAR (2007)**
Evaluation by the RMS (2019): **Relevant and reliable.**

Report KMA 2.5 – Tanada, Y., Omi, E.M. (1974). Persistence of Insect Viruses in Field Populations of Alfalfa Insects, Journal of Invertebrate Pathology, 23, 360-365
Published report
https://doi.org/10.1016/0022-2011(74)90102-5
BVL no 3682711

Abstract: The persistence of viruses of five insects was observed in alfalfa fields. The insects were *Autographa californica, Colias eurytheme, Pseudaletia unipuncta, Spodoptera exigua*, and *Trichoplusia ni*. The isolated viruses were the granulosis (GV), the cytoplasmic-polyhedrosis (CPV), and the nuclear-polyhedrosis (NPV) viruses. The viruses persisted in the soil, on the alfalfa foliage, and in alternate hosts. In the soil, the viruses persisted even during the winter months when no foliage remained on the plants. Alfalfa sprouts harboring virus-infected larvae of *C. eurytheme* and *S. exigua* produced virus infections in larvae of these insects, but those with larvae of *A. californica* and *P. unipuncta* did not cause virus infection. The GVs and CPVs isolated from these insects were transmitted to nearly all of the other four species, but the NPVs appeared to be host specific.

Previous evaluation:	**in DAR (2007)**
Evaluation by the RMS (2019):	**Supplementary information.**

Report KMA 2.5 – Thomas, E.D., Reichelderfer, C.F., Heimpel, A.M. (1973). The Effect of Soil pH on the Persistence of Cabbage Looper Nuclear Polyhedrosis Virus in Soil, Journal of Invertebrate Pathology, 21, 21-25
Published report
https://doi.org/10.1016/0022-2011(73)90108-0
BVL no 3682710

Abstract: The persistence of singly embedded *Trichoplusia ni* nuclear polyhedrosis virus in Norfolk A loamy sand of various pH was studied under laboratory conditions. Virus extracts of the treated soils were bioassayed at three monthly intervals and showed that virus residues are affected by soil pH. Within the range of soil pH tested (4.83-7.17), the lower the pH, the more rapidly the virus was inactivated. The practice of liming fields for maintaining favorable physiological characteristics as well as for nutritional reasons, enhances the persistence of the virus in the soil.

Previous evaluation:	**in DAR (2007)**
Evaluation by the RMS (2019):	**Relevant in regard to baculoviruses in general but less relevant in regard to CpGV in particular.**

Report KMA 2.5 – Evans, H.F., Harrap, K.A. (1982), Persistence of Insect Viruses, In: Mahy, B.W.J., Minson, A.C., and Darby, G.K. (eds) Virus persistence: Thirty-third Symposium of the Society for General Microbiology held at the University of Cambridge, March 1982, pp. 57-96, Cambridge University Press, Cambridge
Published report
3714767

Abstract: One of the features which influences the interaction of insects and their viruses is the discontinuity of the host population. Most insects have a dormant phase where metabolic activity is extremely low and this stage is frequently unavailable for virus infection. In such circumstances the virus has to persist in an infective state until the host is once more available for infection. Persistence of insect viruses therefore means their survival in the natural environment as in most instances insect viruses do not require intimate host-to-host transfer to retain viability. The means by which insect viruses can persist in nature are important both in the initiation and maintenance of infection in a population. The two key features of this persistence are the preservation of virus infectivity and the ways in which transmission to a susceptible host can occur. These are influenced by many factors and their interrelationship is examined in this chapter.

Previous evaluation:	**in DAR (2007)**
Evaluation by the RMS (2019):	**Relevant and reliable but due to publication date possibly not reflecting the current state of knowledge.**

B.2.6 Relationships to known plant or animal or human pathogens

The following information, highlighted in grey, is derived from the Draft Assessment Report Volume 3, Annex B-2, Point B.2.1.6.

Known baculoviruses have been exclusively isolated from arthropods (OECD, 2002, BVL no 3728852) and not from other animals, humans or plants.

CpGV as well as all other baculoviruses are not related to any known plant, animal (other than arthropods) or human pathogen. The host range of baculoviruses in general is restricted to arthropods, and the

host range of granuloviruses is even narrower, restricted to Lepidoptera, with only few lepidopteran species being infected by a single Betabaculovirus species. Replication in animals other than arthropods or in plants was never observed for baculoviruses. No adverse effects on human health has been observed indicating that the use of baculovirus is safe and does not cause any health hazards (OECD, 2002).

New data 2016

No new data have been submitted under this point. The notifier considers the previously submitted information to be acceptable to cover current requirements. No additional references were identified by the notifier from peer-reviewed open literature to be relevant in regard to the data point "Relationships to known plant or animal or human pathogens". Please refer to the "References relied on" section (chapter B.2.10) for a summary and an evaluation of the literature search.

B.2.6.1 Conclusion by RMS

According to the data requirements laid down by Commission Regulation (EU) No 283/2013 (Annex Part B Micro-organisms including viruses) the following points need to be addressed in chapter B.2.6:

"The possible existence of one or more species of the genus of the active and/or, where relevant, contaminating micro-organisms known to be pathogenic to humans, animals, crops or other non-target species and the type of disease caused by them shall be indicated. It shall be stated whether it is possible, and if so, by which means to clearly distinguish the active micro-organism from the pathogenic species."

While it is true that CpGV is not related to known plant, animal or human pathogens with the exception of several other baculoviruses infecting insects of the order *Lepidoptera*, *Hymenoptera*, and *Diptera*, these exceptions could and should have been mentioned here. A list of species in the genus *Betabaculovirus* (to which CpGV belongs) can be found in Herniou *et al.* (2011) and on the webpage of the International Committee on Taxonomy of Viruses – ICTV) under https://talk.ictvonline.org/taxonomy. The most recent summary of the ICTV on Baculoviridae phylogeny (Harrison *et al.*, 2018) is provided at www.ictv.global/report/baculoviridae.

Herniou, E. A., Arif, B. M., Becnel, J. J., Blissard, G. W., Bonning, B., Harrison, R., Jehle, J. A., Theilmann, D. A. and Vlak, J. M., Baculoviridae. In: King, A. M. Q., Adams, M. J., Carstens, E. B., Lefkowitz, E. J. (editors). *Virus Taxonomy*. Oxford: Elsevier; 2011. pp. 163–174.

Harrison, R. L., Herniou, E. A., Jehle, J. A., Theilmann, D. A., Burand, J. P., Becnel, J. J., Krell, P. J., van Oers, M. M., Mowery, J. D., Bauchan, G. R., and ICTV Report Consortium (2018). ICTV Virus Taxonomy Profile: *Baculoviridae*, *Journal of General Virology* 99: 1185-1186.

B.2.6.2 Cited references

Report KMA 2.6 – OECD (2002), Consensus Document on Information used in the Assessment of Environmental Applications involving *Baculovirus* (ENV/JM/MONO(2002)1), Series on Harmonization of Regulatory Oversight in Biotechnology, No.20, OECD Environment Directorate, Paris. (Available on the Biotrack website at http://www.oecd.org/biotrack/)
Published report
BVL no 3728852

Abstract: The OECD's Working Group on Harmonization of Regulatory Oversight in Biotechnology decided at its first session, in June 1995, to focus its work on the development of consensus documents which are mutually acceptable among Member countries. These consensus documents con-

tain information for use during the regulatory assessment of a particular product. This document contains general information on baculoviruses such as organism characteristics, behavior in the environment, their history of use and interactions, as well as environmental safety considerations. Germany served as lead country in the preparation of this document. It has been revised on a number of occasions based on the input from other Member countries. It is intended for use by regulatory authorities and others who have responsibility for assessments and by those who are actively involved with genetic improvement and intensive management of the genus.

Previous evaluation: in DAR (2007)
Evaluation by the RMS (2019): Relevant and reliable.

B.2.7 Genetic stability and factors affecting it

The following information, highlighted in grey, is derived from the Draft Assessment Report Volume 3, Annex B-2, Point B.2.1.7.

The used strain of CpGV was isolated in Mexico and propagated for 9 years at the BBA in Darmstadt/Germany and also for 9 years at Berkeley/California (Harvey and Volkman, 1983, BVL no 3714770). Only one of its enzymatic digests revealed a difference in the DNAs which were subsequently calculated to be 99.94% homologous. No differences were found in molecular weights of enveloped virion polypeptides with SDS-polyacrylamide gel electrophoresis. Also a radioimmune protein blot assay revealed no differences. LD_{50} values for first and fifth instar larvae showed both derivatives to have similar virulence. It is concluded that the used CpGV strain is genetically stable and did not change its properties during all the time under test.

The genetic stability of the CARPOVIRUSINE CpGV isolate produced by NPP (Natural Plant Protection S.A.) was compared with the INRA (National Institute for Agronomic Research) isolate to check the genetic stability during the manufacturing process. In France, the INRA has developed the product CARPOVIRUSINE. Arysta LifeScience S.A.S., formerly CALLIOPE S.A.S., has undertaken the registration and development of the product. The DNAs of each isolate were restricted with *Sal*1, *Eco*R1 and *Bam*H1. The restriction profiles of the reference virus and NPP reference using *Sal*I, *Eco*RI and *Bam*H1 did not show any difference between both isolates (Croizier, 1996, BVL no 2019116).

Biache (1998, BVL no 2019118) compared the CpGV from CARPOVIRUSINE 2000 to the reference isolate from Darmstadt in 1998. Genomes from both isolates were restricted with *Eco*R1 and *Bam*H1 to prove that the NPP isolate is the Mexican isolate that is stable through years of production. The enzymatic restriction profiles obtained did not differ from each other, indicating the molecular identity of the two preparations.

The purified 2001 standard virus from NPP, had not changed compared to NPP 1996 standard. For the enzymes used (*Eco*RI, *Bam*HI, *Sal*I and *Eco*RV), the NPP standard of *Cydia pomonella* virus had the same profile as the *C. pomonella* purified Granulovirus stored in the laboratory since 1971 (Croizier, 2001, BVL no 2388141).

Recently, the isolates used for the production of MADEX and VIRGO and the CpGV isolate obtained from INRA were compared in seperate studies to a reference isolate using DNA Restriction Endonuclease Analysis (REN) (Jehle, 2006, BVL no 3431947). For all enzymes tested, DNA patterns did not show any difference between the MADEX and the INRA isolates and the reference isolate. For three enzymes tested (*Sal*I, *Bam*HI, *Eco*RV), DNA patterns did not show any difference between the Sipcam (now owned by Serbios srl) isolate and the reference isolate. A very faint additional submolar band was observed in the DNA of the test item after digestion with *Eco*RI, indicating a minor variability. Furthermore, comparison with the published restriction profiles of CpGV-M revealed that no differences exist

between the production isolates and the originally described CpGV-M (Jehle 2006), proving that the MADEX, Sipcam (now owned by Serbios srl) and the INRA isolate is the Mexican isolate.

Horizontal gene transfer

The phylogeny of 13 viral species (family Baculoviridae) of the genera Betabaculovirus (formerly Granulovirus) and Alphabaculovirus (formerly Nucleopolyhedrovirus) was reconstructed on the basis of 22 conserved protein families shared by all species, and a comprehensive homology search and phylogenetic analysis of the complete genomes of these viruses was used to test for horizontal gene transfer from cellular organisms. Statistically significant evidence of horizontal transfer was found in the case of six protein families (DNA ligase, ribonucleotide reductase 1, SNF2 global transactivator, inhibitor of apoptosis, chitinase, and UDP-glycosyltransferase). Three of these families are known to play key roles in the infection of insect hosts by these viruses. There was evidence that two of these (inhibitor of apoptosis and UDP-glucosyltransferase) were derived from the insect host. By contrast, the gene encoding chitinase in these viruses was evidently derived from a group of bacteria (the gamma subdivision of proteobacteria), which use chitinase to break down fungal chitins (Hughes and Friedman, 2003, BVL no 2019126).

In very rare cases CpGV may exchange DNA with the host genome as is typical for many if not all viruses during evolutionary time. This is concluded from phylogenetic analyses of different baculovirus genes, which suggest that some of them were acquired from the hosts genome, others from bacteria or other viruses (Hughes and Friedmann, 2003, Herniou et al., 2001, BVL no 3714747) during millions of years. There is also direct evidence for the potential transfer of host DNA sequences to several baculoviruses (OECD, 2002, BVL no 3682775). Intensive screening for CpGV mutants after applying specific infection and selection procedures resulted in the isolation of two CpGV isolates carrying host transposable elements in their genome (Jehle *et al.*, 1995, Jehle *et al.*, 1998). However, it was demonstrated that these mutants were effectively out-competed by the wildtype CpGV-M (Mexican isolate) and would not be able to establish in a mixture together with CpGV-M (Arends *et al.*, 2005).

In conclusion, genetic exchange of virus sequences with other organisms is a natural occurring process, which is unlikely to pose a risk to the environment or human health. Though it cannot be excluded that single virus particle may contain host DNA sequences, the recorded stability of the CpGV genome provides clear evidence that these mutants are extremely seldom and do not establish during the production process (Jehle, 2007, BVL no 1693368).

New data 2016

The notifier considers the previously submitted information to be acceptable to cover current requirements.

A literature search according to EFSA (2011)[9] was conducted by the notifier to identify relevant recent published peer reviewed references covering the last 10 years (Anonymous, 2016, BVL no 3306440). The literature search was conducted on the Scopus database. The data requirement “Biological properties of the micro-organism” was covered using a focused search encompassing baculoviruses in general but focused on specific search terms related to biological properties.This focused search retrieved a large number of references (665) which were sorted manually by the notifier for relevance for the data requirements. After a first check for relevance, 22 references were submitted to full text analysis. According to the full text analysis the notifier regarded 18 references relevant for MA Section 2 and Section 3 of the RAR. Regarding Section 2, only two references were identified as relevant by the notifier. For all details on the selection process and a summary and an evaluation of the literature search by RMS, please refer to the “References relied on” section (chapter B.2.10).
From peer reviewed open literature, no new findings on the genetic stability were identified by the notifier. Instead, references confirming previous articles are presented.

9 Guidance of EFSA: Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009. EFSA Journal 2011;9(2):2092

The two articles found relevant by the notifier examined the alphabaculovirus *Autographa californica* nucleopolyhedrovirus (AcMNPV), which among all baculoviruses has been studied most intensively and consists of highly diverse isolates infecting a rather broad range of lepidopteran species compared to CpGV (Chateigner *et al.*, 2015, BVL no 3714818). As demonstrated by Chateigner *et al.* (2015), occlusion bodies of this particular baculovirus already consist of a large genomic variation in their occluded virions. In contrast to all alphabaculoviruses, virions of betabaculoviruses are occluded singly. Hence, in contrast to AcMNPV genetic diversity of CpGV is restricted within the physical units, the occlusion bodies (Harvey and Volkman, 1983). In general, the genetic diversity reflected by small nuclear point mutations and/or insertions and deletions into the baculovirus genomes, can be considered as part of the co-evolution of baculoviruses and hosts. In this context, *Cydia pomonella* granulovirus also harbours genomic variations that can be accounted to certain lineages of CpGV breaking resistances in codling moth populations (Gueli Alletti *et al.*, 2017, BVL no 3714820). However, the interaction between viruses and their hosts cannot be fully explained by only detecting these genetic variations and molecular characterizations have been mainly conducted with lepidopteran cell cultures and not with the natural hosts. Moreover, the fixation of genetic variants in a baculovirus population is subject to evolution processes that require several generations of infections in hosts.

In a recent article by Gilbert *et al.* (2016, BVL no 3306441) the influx of genetic material from hosts to virus populations was studied. Genomes of the AcMNPV baculovirus were analyzed to identify host DNA sequences integrated in their genomes. Additionally, the frequency of insertions of host DNA into viral genomes was estimated. Based on these data it was calculated that on average 4.8% of viruses harbor at least one moth sequence. Moreover, it was also found that no insertion of moth DNA was maintained in any viral population after 10 successive infection cycles. Yet, Gilbert *et al.* (2016) found that at least 21 of the moth transposable elements integrated into viral genomes underwent repeated horizontal transfers between various insect species, including some lepidopterans susceptible to baculoviruses. These results identify host DNA influx as a potent source of genetic diversity in viral populations. They also support a role for baculoviruses as vectors of DNA horizontal transfer between insects, and call for an evaluation of possible gene spread or spread of transposable elements when using viruses as biopesticides or gene delivery vectors.

Gilbert *et al.* (2014, BVL no 3714821) showed that multiple copies of two transposable elements of the cabbage looper (*Trichoplusia ni*) transposed *in vivo* into genomes of the baculovirus *Autographa californica multiple nucleopolyhedovirus* (AcMNPV) during caterpillar infections. The authors further demonstrated that both transposable elements underwent recent horizontal transfer between several sympatric moth species (*T. ni*, *Manduca sexta*, *Helicoverpa spp.*) showing different degrees of susceptibility to AcMNPV. Although these results provide strong support for the role of viruses as vectors of horizontal transfer of transposable elements between animals, (Gilbert *et al.*, 2014) influx of host DNA into virus genomes and transfer of DNA is already well known, happening constantly, and is part of the natural biological process of viruses. Moreover, it should be pointed out that viruses can only capture DNA from their hosts. Nevertheless, Gilbert *et al.* (2014) call for a systematic evaluation of the frequency and impact of virus-mediated horizontal transfer on the evolution of host genomes. Notably, the insects in which Gilbert *et al.* (2014) identified recent horizontal transfer of transposable elements are agricultural pests that have undergone recent demographic expansion with the intensification of agricultural practices. Baculovirus zoonoses occur naturally in the field and are increasingly exploited for the biological control of these pests. Given the frequency at which baculoviruses potentially shuttle genetic material between host species, it would be relevant to assess the impact of intensive agriculture on the recent evolution of insects (Gilbert *et al.*, 2014).

B.2.7.1 Conclusion by the RMS (2019)

According to Commission Regulation (EU) No 283/2013 (Annex Part B Micro-organisms including viruses) two points need to be addressed in chapter B.2.7. These data requirements are outlined below followed by a short evaluation whether or not they have been adequately addressed by the notifier.

(i) "Where appropriate, information on genetic stability (e.g. mutation rate of traits related to the mode of action or uptake of exogenous genetic material) under the environmental conditions of proposed use must be provided."

The notifier presents information which strongly suggests that the Mexican CpGV isolate is genetically stable over several investigated intervals as profound genetic mutations became not evident within nine years (Harvey and Volkman, 1983) and within five years of continuous propagation (Croizier, 2001). Furthermore, the isolates used in the products Carpovirusine, Madex, and Virgo did not change genetically compared to the originally described Mexican isolate CpGV-M. RMS considers this data requirement as fulfilled.

(ii) "Information must also be provided on the micro-organism's capacity to transfer genetic material to other organisms as well as its capacity to being pathogenic for plants, animals or man. If the micro-organism carries relevant additional genetic elements, the stability of the encoded traits shall be indicated."

The notifier presents information showing that horizontal transfer of genes and transposable elements has been occuring frequently within baculoviruses and indicating a role for baculoviruses as vectors of horizontal DNA transfer between insects. Notably, Gilbert *et al.* (2016) call for an evaluation of possible gene spread or spread of transposable elements when using viruses as biopesticides or gene delivery vectors. RMS would like to point out that the following reports have not been provided by the notifier: Jehle *et al.* (1995), Jehle *et al.* (1998), and Arends *et al.* (2005).

Even though the notifier performed a literature search resulting in two relevant publications in regard to chapter B.2.7 "Genetic stability and factors affecting it" the literature search cannot be regarded as comprehensive. The only specific search term in regard to genetic stability included in the search strategy was "stability". However, this term did not result in the two Gilbert *et al.* papers (2014 and 2016) as "stability" does neither occur in the title, abstract nor keyword of these papers. The reasons why these two papers were found was due to the search term "host" which occurs in both papers in the abstract and in Gilbert *et al.* (2016) also in the title. Therefore, the finding of the Gilbert *et al.* papers is rather accidental than the the result of a focused search as "host" is not necessarily a search term specific for chapter B.2.7. As became already evident with the DAR horizontal gene transfer and the occurrence of transposable elements are apparently factors effecting the genetic stability of baculoviruses in general and CpGV in particular. Therefore, to cover the data requirements concerning chapter B.2.7 the literature search should also have included specific search terms like "horizontal transfer" and "transposable elements" or other specific terms that could in any way be relevant for the virus's capacity to transfer genetic material to other organisms.

B.2.7.2 Cited references

Report KMA 2.7 – Harvey, J. P., Volkman, L. E. (1983). Biochemical and Biological Variation of *Cydia pomonella* (Codling Moth) Granulosis Virus, Virology, 124, 21-34
Published report
https://dx.doi.org/10.1016/0042-6822(83)90287-8
BVL no 3714770

Abstract: *Cydia pomonella* granulosis viruses (CpGV) from three different sources were compared biochemically and biologically. Restriction enzyme profiles of a CpGV isolated in Mexico and propagated for 9 years in a laboratory in Darmstadt, Germany (CpGV-MD) were compared with those of the same isolate propagated for 9 years at a Berkeley, California, laboratory (CpGV-MB). Only one of six enzymatic digests revealed a difference in the DNAs, which were subsequently calculated to be 99.94% homologous. No differences were found in molecular weights of enveloped virion polypeptides with SDS-polyacrylamide gel electrophoresis. In contrast, the restriction profiles of a CpGV isolated in Russia (CpGV-R) differed from that of CpGV-MB and CpGV-MD in five of six enzymatic digests due to what appeared to be a deleted fragment of DNA of about 1×10^6 in the CpGV-R genome.

SDS-PAGE indicated a small difference in mobility of a high-molecular-weight protein in CpGV-R as compared to CpGV-MB and CpGV-MD. Two low-molecular weight proteins present in both CpGV-MD and CpGV-MB were missing completely in CpGV-R. Southern blot hybridization of restriction endonuclease gels using ^{32}P-labeled CpGV-MB DNA showed homology for all three isolates. A radioimmune protein blot assay revealed no differences in serological reactivity among the three CpGVs. LD_{50} values for first and fifth instar larvae showed CpGV-MB and CpGV-MD to have similar virulence, while CpGV-R had significantly less.

Previous evaluation: **in DAR (2007)**
Evaluation by the RMS (2019): **Relevant and reliable.**

Report KMA 2.7 – Croizier, G. (1996). Analyse des virus de la granulose de *Carpocapsa pomonella* contenus dans un culot isolé par centrifugation à 15000g de CHAD filtré. Origine NPP, Arysta LifeScience S.A.S., URA n°2209, Institut National de la Recherche Agronomique, France
unpublished
BVL no 2019116

Abstract: Receipted material, under past form and supply by mail from NPP was diluted in water. The aqueous suspension deposed on a glycerol gradient allowed to separe the numerous granules in the centrifugat material which had a high density. The granules issued from the gradient were dissolved in alcalin medium. Viral DNA after phenolic extraction and alcohol precipitation was identified par restriction analysis using as reference the standard Granulosis virus conserved by the biological control Laboratory of INRA located in La Minières (This standard corresponds to the Mexican isolate replicated in Germany; Harvey & Volkman, Virol. 1983, 124, 21-34). Restriction profils of standard virus and of N.P.P. virus do not allow to show profil differences between the two virus using the 3 restriction enzymes SalI, EcoRI and BamHI. No particular deviation was noted for the analysed sample of granulosis virus of C. pomonella in relation to the standard defining the Carpovirusine identity (characteristic).

Previous evaluation: **in DAR (2007)**
Evaluation by the RMS (2019): **Relevant and reliable.**

Report KMA 2.7 – Biache, G. (1998). Comparison of CpGV isolate from Carpovirusine 2000 with Darmstadt isolate, Arysta LifeScience S.A.S., Institut National de la Recherche Agronomique, France
unpublished
BVL no 2019118

Abstract: After arrival of the formulation and multiplication on codling moths caterpillars, we proceeded to control the genetic stability. The comparison was carried out with the international reference preparation distributed by the B.B.A. (Dr. Huber, Darmstadt), by means of an agarose gel electropherogram after digestion with the endonucleases EcoR1 and BamH1. The enzymatic restriction profiles obtained with Carpovirusine 2000 (photo attached) do not differ from those of the reference preparation; they highlight the molecular identity of the two preparations.

Previous evaluation: **in DAR (2007)**
Evaluation by the RMS (2019): **Relevant and reliable.**

Report KMA 2.7 – Croizier, G. (2001). *Carpocapsa pomonella* granulosis virus analysis. NPP 1996 standard and NPP 2001 standard, Arysta LifeScience S.A.S., EP01630, Institut National de la Recherche Agronomique, France
unpublished
BVL no 2388141

Abstract: Digested DNAs corresponding to NPP virus genomes named 1996 standard and 2001 standard did not show any difference in restriction profiles for visible fragments size range above 1000

nucleotides. The absence of differences was observed with 4 enzymes which offered spread and clearly visible profiles. The two standards, 1996 NPP and 2001 NPP, could not be differentiated from a reference *Carpocapsa pomonella* granulosis virus conserved at Saint Christol since 1971. The purified 2001 standard virus from NPP, according to the analysis criterion retained, had not changed compared to NPP 1996 standard. For the enzymes used, the NPP standard of *Carpocapsa pomonella* virus had the same profile as the *Carpocapsa pomonella* purified granulosis virus in my laboratory since 1971.

Previous evaluation: **in DAR (2007)**
Evaluation by the RMS (2019): **Relevant and reliable.**

KMA 2.7 – Jehle, J. (2006), Comparative Restriction Analysis CpGV (Neustadt Mexican isolate) with CpGV (Madex Mexican isolate)
Unpublished
BVL no 3431947

Summary: For the identification of baculovirus isolates DNA endonuclease restriction (REN) analysis is usually used. By digesting viral DNA by different RENs specific restriction patterns can be identified and small genotypic variations can be located in a restriction map. In this study, viral DNAs of CpGV (Mexican strain, Neustadt) (reference item) and CpGV (Madex Mexican strain) (test item) were isolated and purified and subjected to endonuclease restriction analysis using the endonucleases *Sal*I, *Bam*HI, *Eco*RI and *Eco*RV. The restriction fragments were separated in an agarose gel and the obtained restriction profiles were compared to each other and to published profiles of CpGV-M. It was found that the restricton profiles of both items did neither differ from each other nor to published restriction profiles of CpGV-M. It can be concluded that the test item CpGV (Madex Mexican strain is identical to CpGV-M.

Previous evaluation: **in DAR (2007)**
Evaluation by the RMS (2019): **Relevant and reliable.**

Report KMA 2.7 – Hughes, A. L. and Friedman, R. (2003), Genome-Wide Survey for Genes Horizontally Transferred from Cellular Organisms to Baculoviruses, Molecular Biology and Evolution, 20, 979–987
published report
http://dx.doi.org/10.1093/molbev/msg107
BVL no 2019126

Abstract: The phylogeny of 13 viral species in the genera *Granulovirus* and *Nucleopolyhedrovirus* (family Baculoviridae) was reconstructed on the basis of 22 conserved protein families shared by all species, and a comprehensive homology search and phylogenetic analysis of the complete genomes of these viruses was used to test for horizontal gene transfer from cellular organisms. Statistically significant evidence of horizontal transfer was found in the case of six protein families (DNA ligase, ribonucleotide reductase 1, SNF2 global transactivator, inhibitor of apoptosis, chitinase, and UDP-glucosyltransferase). Three of these families are known to play key roles in the infection of insect hosts by these viruses. There was evidence that two of these (inhibitor of apoptosis and UDP-glucosyltransferase) were derived from the insect host. By contrast, the gene encoding chitinase in these viruses was evidently derived from a group of bacteria (the gamma subdivision of proteobacteria), which use chitinase to break down fungal chitins.

Previous evaluation: **in DAR (2007)**
Evaluation by the RMS (2019): **Relevant and reliable.**

Report KMA 2.7 – Herniou, E. A., Luque, T., Chen, X., Vlak, J. M., Winstanley, D., Cory, J. S. and O'Reilly, D. R. (2001), Use of Whole Genome Sequence Data To Infer Baculovirus Phylogeny, J. Virol., 75, 8117-8126
published report
http://dx.doi.org/10.1128/JVI.75.17.8117-8126.2001
BVL no 3714747

Abstract: Several phylogenetic methods based on whole genome sequence data were evaluated using data from nine complete baculovirus genomes. The utility of three independent character sets was assessed. The first data set comprised the sequences of the 63 genes common to these viruses. The second set of characters was based on gene order, and phylogenies were inferred using both breakpoint distance analysis and a novel method developed here, termed neighbor pair analysis. The third set recorded gene content by scoring gene presence or absence in each genome. All three data sets yielded phylogenies supporting the separation of the *Nucleopolyhedrovirus* (NPV) and *Granulovirus* (GV) genera, the division of the NPVs into groups I and II, and species relationships within group I NPVs. Generation of phylogenies based on the combined sequences of all 63 shared genes proved to be the most effective approach to resolving the relationships among the group II NPVs and the GVs. The history of gene acquisitions and losses that have accompanied baculovirus diversification was visualized by mapping the gene content data onto the phylogenetic tree. This analysis highlighted the fluid nature of baculovirus genomes, with evidence of frequent genome rearrangements and multiple gene content changes during their evolution. Of more than 416 genes identified in the genomes analyzed, only 63 are present in all nine genomes, and 200 genes are found only in a single genome. Despite this fluidity, the whole genome-based methods we describe are sufficiently powerful to recover the underlying phylogeny of the viruses.

Previous evaluation: **in DAR (2007)**
Evaluation by the RMS (2019): **Relevant and reliable.**

Report KMA 2.1.1 – OECD (2002), Consensus Document on Information used in the Assessment of Environmental Applications involving *Baculovirus* (ENV/JM/MONO(2002)1), Series on Harmonization of Regulatory Oversight in Biotechnology, No.20, OECD Environment Directorate, Paris. (Available on the Biotrack website at http://www.oecd.org/biotrack/)
Published report
BVL no 3682775

Abstract: The OECD's Working Group on Harmonization of Regulatory Oversight in Biotechnology decided at its first session, in June 1995, to focus its work on the development of consensus documents which are mutually acceptable among Member countries. These consensus documents contain information for use during the regulatory assessment of a particular product. This document contains general information on baculoviruses such as organism characteristics, behavior in the environment, their history of use and interactions, as well as environmental safety considerations. Germany served as lead country in the preparation of this document. It has been revised on a number of occasions based on the input from other Member countries. It is intended for use by regulatory authorities and others who have responsibility for assessments and by those who are actively involved with genetic improvement and intensive management of the genus.

Previous evaluation: **in DAR (2007)**
Evaluation by the RMS (2019): **Relevant and reliable.**

Report KMA 2.7 – Jehle, J. A., Fritsch, E., Nickel, A., Huber, J., Backhaus, H. (1995), TCl4.7: A Novel Lepidopteran Transposon Found in *Cydia pomonella* Granulosis Virus, Virology, 207, 369-379
published report
http://dx.doi.org/10.1006/viro.1995.1096

Abstract: After the co-infection of larvae of the lepidopteran *Cryptophlebia leucotreta* with the two baculoviruses *C. leucotreta* granulosis virus and *Cydia pomonella* granulosis virus (ClGV and CpGV, respectively), three CpGV mutants and one ClGV mutant carrying insertions of 0.9 to 4.7 kb have been isolated. By cloning, sequencing, and hybridization analysis, one of these insertions was identified as a transposon-like element derived from the *C. leucotreta* genome. This element, called TCl4.7, was found in the genome of CpGV which naturally replicates in *C. pomonella*. Sequence analysis suggested that TCl4.7 is 4726 bp in size, flanked by imperfect inverted terminal repeats of 29 bp, and integrated into the target dinucleotide TA. TCl47 encompasses an open reading frame sharing homologies to transposase genes of the Tc1-related transposable elements found in *Caenorhabditis* and in *Drosophila* species. The open reading frame might represent a pseudogene since it is missing an ATG start codon. The integration site of TCl4.7 is located in a non-protein-coding region of the CpGV genome at m.u. 9.5. In bioassays the TCl4.7-carrying virus and all the other mutants except for one showed LC_{50} values similar to those of CpGV and ClGV. This is the first report of the horizontal escape of a transposable element during the *in vivo* infection of lepidopteran larvae by granulosis viruses.

Previous evaluation: **in DAR (2007)**
Evaluation by the RMS (2019): **Relevant and reliable. Please note that in the DAR this paper was cited as personally communicated by Jehle in 2007. A copy of the publication was not provided by the notifier.**

Report KMA 2.7 – Jehle, J. A., Nickel, A., Vlak, J. M. Backhaus, H. (1998), Horizontal Escape of the Novel Tc1-Like Lepidopteran Transposon TCp3.2 into *Cydia pomonella* Granulovirus, Journal of Molecular Evolution 46, 215–224
published report
http://dx.doi.org/10.1007/PL00006296

Abstract: We characterized an insertion mutant of the baculovirus *Cydia pomonella* granulovirus (CpGV), which contained a transposable element of 3.2 kb. This transposon, termed TCp3.2, has unusually long inverted terminal repeats (ITRs) of 756 bp and encodes a defective gene for a putative transposase. Amino acid sequence comparison of the defective transposase gene revealed a distant relationship to a putative transposon in *Caenorhabditis elegans* which also shares some similarity of the ITRs. Maximum parsimony analysis of the predicted amino acid sequences of Tc1- and mariner-like transposases available from the GenBank data base grouped TCp3.2 within the superfamily of Tc1-like transposons. DNA hybridization indicated that TCp3.2 originated from the genome of *Cydia pomonella,* which is the natural host of CpGV, and is present in less than 10 copies in the *C. pomonella* genome. The transposon TCp3.2 most likely was inserted into the viral genome during infection of host larvae. TCp3.2 and the recently characterized Tc1-like transposon TC14.7 (Jehle et al. 1995), which was also found in a CpGV mutant, represent a new family of transposons found in baculovirus genomes. The occasional horizontal escape of different types of host transposons into baculovirus genomes evokes the question about the possible role of baculoviruses as an interspecies vector in the horizontal transmission of insect transposons.

Previous evaluation: **in DAR (2007)**
Evaluation by the RMS (2019): **Relevant and reliable. Please note that in the DAR this paper was cited as personally communicated by Jehle in 2007. A copy of the publication was not provided by the notifier.**

Report KMA 2.7 – Arends, H. M., Winstanley, D., and Jehle, J. A. (2005), Virulence and competitiveness of *Cydia pomonella* granulovirus mutants: parameters that do not match, Journal of General Virology, 86, 2731–2738
published report
http://dx.doi.org/10.1099/vir.0.81152-0

Abstract: The LD_{50}, median survival time (ST_{50}) and virus production are virulence parameters that are commonly used to describe the biological characteristics of viruses. In this study, these parameters were determined for *Cydia pomonella granulovirus* (CpGV-M) and two naturally occurring mutants (CpGV-MCp4 and -MCp5) that carry Tc1-like insect transposable elements. The three virus genotypes were similar in their LD_{50}, ST_{50} and virus production. However, the mutant genotypes MCp4 and MCp5 were very effectively out-competed by CpGV-M in direct competition experiments, where *Cydia pomonella* larvae were co-infected with known ratios of occlusion bodies or budded virus of CpGV-M and one of the two mutants. It was demonstrated that MCp5 and MCp4 could not be sustained in the virus population when the progeny viruses of different co-infections were used as inocula to infect next passage larvae. These results show that the virulence parameters LD_{50}, ST_{50} and virus production alone do not adequately reflect the competitiveness of the virus and are thus not suitable to describe virus population dynamics.

Previous evaluation: **in DAR (2007)**
Evaluation by the RMS (2019): **Relevant and reliable. Please note that in the DAR this paper was cited as personally communicated by Jehle in 2007. A copy of the publication was not provided by the notifier.**

Report KMA 2.7 – Jehle, J. (2007), Communication Johannes Jehle, DLR, Arysta LifeScience S.A.S.
unpublished
BVL no 1693368

Abstract: The active ingredient consists of the Mexican isolate of CpGV (CpGV-M), which was discovered in Mexico in 1963. There are only a few other CpGV isolates described in the literature, e.g. in 1974, the isolate CpGV-R was obtained from a field-collected larva in Russia and isolate CpGV-E was obtained in England from diseased larvae in a laboratory reared stock of *C. pomonella* at the University of Reading. All viruses consist of a mixture of slightly differing genotypes. As demonstrated by *in vivo* cloning and restriction analyses, CpGV-M is a highly homogenous composition of virus genotypes. CpGV-M has a genome of about 123.5 kb is can be considered as extremely stable. DNA restriction endonuclease (REN) profiles of CpGV-M, which were independently propagated for nine years in laboratories in Darmstadt, Germany (CpGV-MD) and in Berkeley, California (CpGV-MB) revealed a singular difference in their REN profiles in only one of six digests suggesting a genomic identity of 99.9%. Hence, CpGV can be considered as a very stable virus. CpGV-MD and CpGV-MB had similar mean lethal dose (LD_{50}) values for first and fifth instar larvae of *C. pomonella*, respectively, those of CpGV-R were significantly lower. By comparing seven different sources of CpGV from Europe, America and New Zealand by REN analysis it was found that most samples were indistinguishable from CpGV-M, whereas the CpGV-R and CpGV-E had small genotypic differences. In very rare cases CpGV may exchange DNA with the host genome as it is typical for many if not all viruses during evolutionary time. This is concluded from phylogenetic analyses of different baculovirus genes, which suggest that some of them were acquired from the hosts´ genome, others from bacteria or other viruses during millions of years. There is also direct evidence for the potential transfer of host DNA sequences to several baculoviruses. Intensive screening for CpGV mutants after applying specific infection and selection procedures resulted in the isolation of two CpGV isolates carrying host transposable elements in their genome. However, it was demonstrated that these mutants were effectively out-competed by the wildtype CpGV-M and would not be able to establish in a mixture together with CpGV-M. In conclusion, genetic exchange of virus sequences with other organisms is a natural occurring process, which is extremely unlikely to pose a risk to the environment or human health. Though it cannot be excluded that single virus particle may contain host DNA sequences, the recorded

stability of the CpGV genome provides clear evidence that these mutants are extremely seldom and do not establish during the production process.

Previous evaluation: in DAR (2007)
Evaluation by the RMS (2019): Relevant and reliable.

Report KMA 2.7/01 – Anonymous (2016), Literature review report on *Cydia pomonella* Granulovirus biological properties
Not published
BVL no 3306440

Summary: The aim of this report is to provide a global overview of peer-reviewed literature concerning side effects of the active substance, *Cydia pomonella* Granulovirus. This active substance, *Cydia pomonella* Granulovirus, belongs to the family of *Baculoviridae*. Within this taxonomic group *Cydia pomonella* Granulovirus belongs to the genus of Betabaculovirus, which comprises lepidopteran-specific Granuloviruses. Data requirements encompassed in this report are "Biological properties of the micro-organism". Separate reports are dedicated to "Effects on non-target organisms", "Fate and behaviour in the environment", "Effects on human health" and "Residues in or on treated products, food and feed". The data requirement "Biological properties of the micro-organism" was covered using a focused search encompassing baculoviruses in general but focused on specific search terms related to biological properties. This focused search retrieved a large number of references (665) which were sorted manually for relevance for the data requirements based on the following criteria: (1) The article concerns a baculovirus (other viruses are not included) which has not been genetically modified, (2) The article concerns the data requirement "Biological properties of the micro-organism ": genetic stability, resistance, host specificity and mode of action. For completeness purpose, following additional subjects were included: Environmental factors affecting efficacy and virus physiology not directly related to efficacy, (3) Articles concerning efficacy of Baculovirus against mosquitoes were excluded, (4) Articles concerning host specificity, which had already been retrieved during the search concerning Ecotoxicology, were not considered again. In this manner, 22 references were selected for this data requirement.

Submitted for the purpose of renewal
Evaluation by the RMS (2019): Relevant but not comprehensive.

Report KMA 2.7/02 – Gilbert, C., Peccoud, J., Chateigner, A., Moumen, B., Cordaux, R., Herniou, E.A (2016), Continuous Influx of Genetic Material from Host to Virus Populations, PLoS Genetics 12: e1005838.
published report
http://dx.doi.org/10.1371/journal.pgen.1005838
BVL no 3306441

Summary: Many genes of large double-stranded DNA viruses have a cellular origin, suggesting that host-to-virus horizontal transfer (HT) of DNA is recurrent. Yet, the frequency of these transfers has never been assessed in viral populations. Here we used ultra-deep DNA sequencing of 21 baculovirus populations extracted from two moth species to show that a large diversity of moth DNA sequences (n = 86) can integrate into viral genomes during the course of a viral infection. The majority of the 86 different moth DNA sequences are transposable elements (TEs, n = 69) belonging to 10 superfamilies of DNA transposons and three superfamilies of retrotransposons. The remaining 17 sequences are moth sequences of unknown nature. In addition to bona fide DNA transposition, we uncover microhomology-mediated recombination as a mechanism explaining integration of moth sequences into viral genomes. Many sequences integrated multiple times at multiple positions along the viral genome. We detected a total of 27,504 insertions of moth sequences in the 21 viral populations and we calculate that on average, 4.8% of viruses harbor at least one moth sequence in these populations. Despite this substantial proportion, no insertion of moth DNA was maintained in any viral population after 10 successive infection cycles. Hence, there is a constant turnover of host DNA inserted

into viral genomes each time the virus infects a moth. Finally, we found that at least 21 of the moth TEs integrated into viral genomes underwent repeated horizontal transfers between various insect species, including some lepidopterans susceptible to baculoviruses. Our results identify host DNA influx as a potent source of genetic diversity in viral populations. They also support a role for baculoviruses as vectors of DNA HT between insects, and call for an evaluation of possible gene or TE spread when using viruses as biopesticides or gene delivery vectors.

Submitted for the purpose of renewal

Evaluation by the RMS (2019): Relevant and reliable.

Report KMA 2.7/03 – Gilbert, C., Chateigner, A., Ernenwein, L., Barbe, V., Bézier, A., Herniou, E. A. and Cordaux, R. (2014), Population Genomics Supports Baculoviruses as Vectors of Horizontal Transfer of Insect Transposons, Nature Communications, 5, 3348.
published report
http://dx.doi.org/10.1038/ncomms4348
BVL no 3714821

Summary: Horizontal transfer (HT) of DNA is an important factor shaping eukaryote evolution. Although several hundreds of eukaryote-to-eukaryote HTs of transposable elements (TEs) have been reported, the vectors underlying these transfers remain elusive. Here, we show that multiple copies of two TEs from the cabbage looper (*Trichoplusia ni*) transposed *in vivo* into genomes of the baculovirus *Autographa californica multiple nucleopolyhedrovirus* (AcMNPV) during caterpillar infection. We further demonstrate that both TEs underwent recent HT between several sympatric moth species (*T. ni*, *Manduca sexta*, *Helicoverpa spp.*) showing different degrees of susceptibility to AcMNPV. Based on two independent population genomics data sets (reaching a total coverage >330,000X), we report a frequency of one moth TE in ~8,500 AcMNPV genomes. Together, our results provide strong support for the role of viruses as vectors of TE HT between animals, and they call for a systematic evaluation of the frequency and impact of virus-mediated HT on the evolution of host genomes.

Submitted for the purpose of renewal

Evaluation by the RMS (2019): Relevant and reliable.

Report KMA 2.7/04 – Chateigner, A., Bézier, A., Labrousse, C., Jiolle, D., Barbe, V., Herniou, E. A. (2015), Ultra Deep Sequencing of a Baculovirus Population Reveals Widespread Genomic Variations, Viruses, 7, 3625-3646.
published report
http://dx.doi.org/10.3390/v7072788
BVL no 3714818

Summary: Viruses rely on widespread genetic variation and large population size for adaptation. Large DNA virus populations are thought to harbor little variation though natural populations may be polymorphic. To measure the genetic variation present in a dsDNA virus population, we deep sequenced a natural strain of the baculovirus *Autographa californica* multiple nucleopolyhedrovirus. With 124,221X average genome coverage of our 133,926 bp long consensus, we could detect low frequency mutations (0.025%). K-means clustering was used to classify the mutations in four categories according to their frequency in the population. We found 60 high frequency non-synonymous mutations under balancing selection distributed in all functional classes. These mutants could alter viral adaptation dynamics, either through competitive or synergistic processes. Lastly, we developed a technique for the delimitation of large deletions in next generation sequencing data. We found that large deletions occur along the entire viral genome, with hotspots located in homologous repeat regions (hrs). Present in 25.4% of the genomes, these deletion mutants presumably require functional complementation to complete their infection cycle. They might thus have a large impact on the fitness of the baculovirus population. Altogether, we found a wide breadth of genomic variation in the baculovirus population, suggesting it has high adaptive potential.

Submitted for the purpose of renewal	
Evaluation by the RMS (2019):	**Supplementary information.**

Report KMA 2.7/05 – Gueli Alletti, G., Sauer, A. J., Weihrauch, B., Fritsch, E., Undorf-Spahn, K., Wennmann, J. T., Jehle. J. A. (2017), Using Next Generation Sequencing to Identify and Quantify the Genetic Composition of Resistance- Breaking Commercial Isolates of Cydia pomonella Granulovirus, Viruses, 9, 1-16.
published report
http://dx.doi.org/10.3390/v9090250
BVL no 3714820

Summary: The use of Cydia pomonella granulovirus (CpGV) isolates as biological control agents of codling moth (CM) larvae is important in organic and integrated pome fruit production worldwide. The commercially available isolates CpGV-0006, CpGV-R5, and CpGV-V15 have been selected for the control of CpGV resistant CM populations in Europe. In infection experiments, CpGV-0006 and CpGV-R5 were able to break type I resistance and to a lower extent also type III resistance, whereas CpGV-V15 overcame type I and the rarely occurring type II and type III resistance. The genetic background of the three isolates was investigated with next generation sequencing (NGS) tools by comparing their nucleotide compositions to whole genome alignments of five CpGV isolates representing the known genetic diversity of the CpGV genome groups A to E. Based on the distribution of single nucleotide polymorphisms (SNPs) in Illumina sequencing reads, we found that the two isolates CpGV-0006 and CpGV-R5 have highly similar genome group compositions, consisting of about two thirds of the CpGV genome group E and one third of genome group A. In contrast, CpGV-V15 is composed of equal parts of CpGV genome group B and E. According to the identified genetic composition of these isolates, their efficacy towards different resistance types can be explained and predictions on the success of resistance management strategies in resistant CM populations can be made.

Submitted for the purpose of renewal	
Evaluation by the RMS (2019):	**Relevant and reliable.**

B.2.8 Information on the production of metabolites (especially toxins)

The following information, highlighted in grey, is derived from the Draft Assessment Report Volume 3, Annex B-2, Point B.2.1.9.

Viruses have no metabolism of their own and are therefore not able to produce secondary metabolites.

New data 2016
No new data have been submitted under this point. The notifier considers the previously submitted information to be acceptable to cover current requirements. No additional references were identified by the notifier from peer-reviewed open literature to be relevant in regard to the data point "Information on the production of metabolites (especially toxins)". Please refer to the "References relied on" section (chapter B.2.10) for a summary and an evaluation of the literature search.

B.2.8.1 Conclusion by the RMS (2019)

According to Commission Regulation (EU) No 283/2013 (Annex Part B Micro-organisms including viruses) four points need to be addressed in chapter B.2.8. These data requirements are outlined below followed by a short evaluation whether or not they have been adequately addressed by the notifier.

(i) "If other strains belonging to the same microbial species as the strain subject to the application are known to produce metabolites (especially toxins) with unacceptable effects on human health and/or the environment during or after application, the nature and structure of this substance, its presence inside or outside the cell and its stability, its mode of action (including external and internal factors of the micro-organism necessary to action) as well as its effect on humans, animals or other non-target species shall be provided."

The only statement made in regard to this data requirement is that *"viruses have no metabolism of their own and are therefore not able to produce secondary metabolites."* Similarly, the notifier states in the introductory part of Section B.2. "Biological properties of the micro-organism": *"CpGV and the whole family of baculoviruses are not related to any animal (other than arthropods) or plant pathogen and it does not produce any metabolite. For these reasons, no harmful effects from CpGV on humans, other vertebrates, other non-target organisms or the environment are expected."* However, neither statement has been backed up by references. Moreover, neither "metabolite" nor "toxin" were included as specific search terms in the search strategy. Yet, a literature search covering the last ten years would be necessary to maintain the previous evidence that no metabolites and toxins are produced by CpGV isolates.

(ii) "The conditions under which the micro-organism produces the metabolite(s) (especially toxin(s)) must be described."

(iii) "Any available information on the mechanism by which the micro-organisms regulate the production of the(se) metabolite(s) shall be provided."

(iv) "Any available information on the influence of the produced metabolites on the micro-organism's mode of action shall be provided."

If neither metabolites nor toxins are produced by any CpGV isolate under any environmental condition points (ii) – (iv) do not apply. However, as long as no recent references have been provided that this is indeed the case these points cannot be considered as having been addressed and thus remain open.

B.2.8.2 Cited references

None

B.2.9 Antibiotics and other anti-microbial agents

No additional references were identified during the peer-reviewed literature search to address this data point. Since no new data was submitted for this chapter within the renewal process of the active substance existing data have to be used. However, the already presented data fulfils the existing data requirements.

Generally, viruses cannot produce antimicrobial substances because they are not metabolically active. However possible viral resistance to virucidal or virustatic drugs cannot be excluded. In addition transmission of that such resistance from one virus species to another one has not been proven so far.

In the original assessment report (DAR 2007, ASB2010-10675), the following additional statement was made:

"Viruses are sensitive to desinfectants that act by a chemical or physical principle and for which resistance is not a case. Viruses can be also susceptible to virucidal or virustatic drugs acting mostly by inhibiting certain enzymes. Indeed, viruses may develop resistance to these compounds. However, the

mechanism behind the occurrence of viral resistance is different from those in, e.g., bacteria and there is no evidence that resistance may be transmitted from one virus species to another."

B.2.10 References relied on

B.2.10.1 Summary of literature search performed by the notifier

The data requirement "Biological properties of the micro-organism" was covered using a focused literature search. The notifier used the 'Scopus' database considering that:

- this database is known for being one the of the most comprehensive in the field
- an important number of references were retrieved even after removing duplicates (i.e. 665 references)
- manual sorting of the obtained references limited the risk of excluding relevant studies.

The search encompassed baculoviruses in general, including names of commercial products based on CpGV, combined with specific search terms related to biological properties of the microroganism (e.g. resistance, mode of action etc.). To reduce background noise, some specific search terms were excluded from the search. This concerned mainly terms related to the use of Baculovirus as a vector for experimental gene transfer. Details on the used search queries are presented below.

Justification for choosing the source:
Scopus is the largest abstract and citation database of peer-reviewed literature. Scopus delivers the most comprehensive overview of the world's research output in the fields of science, technology, medicine, social sciences and arts and humanities. Updated daily, Scopus contains more than 57 million records including:

- over 21,000 peer-reviewed journals
- articles-in-press (i.e., articles that have been accepted for publication) from more than 5000 international publishers
- 100,000 books
- 520 book series

Date of the search: 24/08/16
Date span of the search: 01/01/2005 to 24/08/16
Date of the latest database update included in the search: 24/08/16

Search strategies used:

{

{

{ [TITLE-ABS-KEY (baculovirus) OR
TITLE-ABS-KEY (baculoviridae) OR
TITLE-ABS-KEY (nucleopolyhedrovirus) OR
TITLE-ABS-KEY (nuclear AND polyhedrosis AND virus) OR
TITLE-ABS-KEY (granulovirus) OR
TITLE-ABS-KEY (betabaculovirus)] }

OR

{ [TITLE-ABS-KEY (cydia AND pomonella AND granulovirus) OR
TITLE-ABS-KEY (cydia AND pomonella AND gv) OR
TITLE-ABS-KEY (cpgv) OR
TITLE-ABS-KEY (cydia AND pomonella AND granulosis virus) OR
TITLE-ABS-KEY (carpovirusine) OR
TITLE-ABS-KEY (virosoft) OR
TITLE-ABS-KEY (granusal) OR
TITLE-ABS-KEY (madex) OR
TITLE-ABS-KEY (virin) OR
TITLE-ABS-KEY (cyap) OR
TITLE-ABS-KEY (carpovirus AND plus) OR
TITLE-ABS-KEY (cyd-x) OR
TITLE-ABS-KEY (carpostop) OR
TITLE-ABS-KEY ("Evo 2") OR
TITLE-ABS-KEY (carpo 600) OR
TITLE-ABS-KEY (virgo AND *virus)] }

AND NOT

[TITLE-ABS-KEY (net present value) OR
TITLE-ABS-KEY (protein expression) OR
TITLE-ABS-KEY (diagnostic test accuracy study) OR
TITLE-ABS-KEY (recombinant proteins) OR
TITLE-ABS-KEY (baculovirus expression system) OR

TITLE-ABS-KEY (gene expression) OR
TITLE-ABS-KEY (predictive value) OR
TITLE-ABS-KEY (predictive value) OR
TITLE-ABS-KEY (predictive value of tests) OR
TITLE-ABS-KEY (diagnostic accuracy) OR
TITLE-ABS-KEY ("Diagnostic value")]

}

AND

PUBYEAR > 2005

}

AND

[TITLE-ABS-KEY (stability) OR
TITLE-ABS-KEY (resistance) OR
TITLE-ABS-KEY (specificity) OR
TITLE-ABS-KEY (host) OR
TITLE-ABS-KEY (mode of action)]

This search strategy retrieved 665 references which were sorted manually for relevance based on the following criteria:

- The article concerns a baculovirus (other viruses are not included) which has not been genetically modified
- The article concerns the data requirement "Biological properties of the micro-organism": genetic stability, resistance, host specificity, and mode of action. For the purpose of completeness the following additional subjects were included: environmental factors affecting efficacy and virus physiology not directly related to efficacy.
- Articles concerning the efficacy of Baculovirus against mosquitoes were excluded.
- Articles concerning host specificity, which had already been retrieved during the search concerning the data requirement “Ecotoxicology”, were not considered again.

This approach retrieved 22 references, of which 4 studies were excluded from further consideration after detailed assessment of relevance. In total 18 studies were considered relevant (or not irrelevant) by the notifier (Table B.2.10-1).

Table B.2.10-1 Results of the study selection process.

	n
Total number of summary records retrieved	665
Number of summary records excluded from search results after rapid assessment of relevance	643
Total number of full-text documents assessed in detail	22
Number of studies excluded from further consideration after detailed assessment of relevance	4
Number of studies not excluded from further consideration after detailed assessment of relevance (i.e. relevant studies and studies of unclear relevance)	**18**

Of the 18 studies generally considered relevant, 3 studies were considered relevant by the notifier for section B.2 “Biological properties of the micro-organism” (Table B.2.10-2). The remaining 15 studies were considered relevant for section B.3 “Further information on the micro-organism”, particularly for chapter B.3.5 “Information on the occurrence or possible occurrence of the development of resistance of the target organism(s)”. Thus, these studies are not listed in Table B.2.10-2Table B.2.10-2) .

Table B.2.10-2 Studies considered relevant to data requirement B.2 after assessment of full text.

Data requirement (numbered according to Regulation 283/2013 part B)	**Author(s)**	**Year**	**Title**	**Source**
2.2.2. Mode of action	Jehle, J. A, Blissard, G. W., Bonning, B. C,, Cory, J. S., Herniou, E. A., Rohrmann, G. F., Theilmann, D. A., Thiem, S. M., Vlak, J. M.	2006	On the classification and nomenclature of baculoviruses: A proposal for revision	Archives of Virology, 151, 1257-1266
2.7. Genetic stability and factors affecting it	Gilbert, C., Chateigner, A., Ernenwein, L., Barbe, V., Bézier, A., Herniou, E. A. and Cordaux, R.	2014	Population genomics supports baculoviruses as vectors of horizontal transfer of insect transposons	Nature communications, 5, 3348
	Gilbert, C., Peccoud, J., Chateigner, A., Moumen, B., Cordaux, R., Herniou, E. A.	2016	Continuous Influx of Genetic Material from Host to Virus Populations	PLoS Genetics,12, e1005838.

B.2.10.2 Evaluation of literature search by the RMS (2019)

As summarized above the notifier performed a focused literature search using a title, abstract, and keyword search. The search encompassed baculoviruses in general, including names of commercial products based on CpGV, combined with specific search terms related to biological properties of the microroganism (i.e.

stability, **resistance**, **specificity**, **host**, and **mode of action**). To reduce background noise, some specific search terms were excluded from the search. This concerned mainly terms related to the use of baculoviruses as a vector for experimental gene transfer. Details on the used search queries have been presented above.

Of the 18 studies generally considered relevant, 3 studies were considered relevant by the notifier for section B.2 "Biological properties of the micro-organism" (Table B.2.10-2). Notably, the publication by Jehle *et al.* (2006) which was considered relevant for chapter B.2.2.2 "Mode of action" was not included in this chapter. This is not surprising as the publication is not related to the mode of action but to the classification and nomenclature of baculoviruses. Therefore, RMS is wondering why this publication was considerd for chapter B.2.2.2 by the notifier in the first place.

Even though the search strategy is extensive in regard to baculoviruses in general it cannot be regarded as comprehensive in regard to the specific search terms related to the data requirements for section B.2 "Biological properties of the micro-organism" as the latter comprise five terms only, i.e. **specificity**, **host**, **mode of action, stability**, and **resistance**. While these terms cover certain data requirements for some chapters (Table B.2.10-3Table B.2.10-3) they do not for others.

Table B.2.10-3: Relevance of the specific search terms used in the literature search for the data requirements concerning section B.2 "Biological properties of the micro-organism"

Specific search term	Relevance for chapter*	Specific data requirement*
specificity	B.2.3 **Host specificity** range and effects on species other than the target harmful organism	Any available information on the effects on non-target organisms within the area to which the micro-organism may spread shall be given. The occurrence of non-target organisms being either closely related to the target species or being especially exposed shall be indicated.
host		Any experience of the toxic effect of the active substance or its metabolic products on humans or animals, of whether the organism is capable of colonising or invading humans or animals (including immunosuppressed individuals) and whether it is pathogenic shall be stated. Any experience of whether the active substance or its products may irritate skin, eyes or respiratory organs of humans or animals and whether it is allergenic in contact with skin or when inhaled shall be stated.
	B.2.1.2 Origin and natural occurrence	The geographical region and the place in the ecosystem (e.g. **host** plant, **host** animal, or soil from which the micro-organism was isolated) must be stated. The method of isolation of the micro-organism shall be reported. The natural occurrence of the micro-organism in the relevant environment shall be given if possible at strain level.
	B.2.2.1 Description of target organisms	Where relevant, details of harmful organisms against which protection is afforded, must be provided.

Specific search term	Relevance for chapter*	Specific data requirement*
	B.2.4 Development stages/life cycle of the micro-organism	Information on the life cycle of the micro-organism, described symbiosis, parasitism, competitors, predators, etc., including **host** organisms, as well as vectors for viruses, must be presented.
	B.2.5 Infectiveness, dispersal and colonisation ability	Information on possible dispersal routes of the micro-organism (via air as dust particles or aerosols, with **host** organisms as vectors, etc.), under typical environmental conditions relevant to the use, must be provided.
mode of action	B.2.2.2 Mode of action	The principal **mode of action** shall be indicated. In connection with the **mode of action** it shall also be stated if the micro-organism produces a toxin with a residual effect on the target organism. In that case, the **mode of action** of this toxin shall be described.
	B.2.8 Information on the production of metabolites (especially toxins)	Any available information on the influence of the produced metabolites on the micro-organism's **mode of action** shall be provided.
	B.2.7 Genetic **stability** and factors affecting it	Where appropriate, information on genetic **stability** (e.g. mutation rate of traits related to the **mode of action** or uptake of exogenous genetic material) under the environmental conditions of proposed use must be provided.
stability		
mode of action	B.2.8 Information on the production of metabolites (especially toxins)	If other strains belonging to the same microbial species as the strain subject to the application are known to produce metabolites (especially toxins) with unacceptable effects on human health and/or the environment during or after application, the nature and structure of this substance, its presence inside or outside the cell and its **stability**, its **mode of action** (including external and internal factors of the micro-organism necessary to action) as well as its effect on humans, animals or other non-target species shall be provided.
stability		
	B.2.5 Infectiveness, dispersal and colonisation ability	The possible effect of factors such as temperature, UV light, pH, and the presence of certain substances on the **stability** of relevant toxins must also be stated.
	B.2.7 Genetic **stability** and factors affecting it	Information must also be provided on the micro-organism's capacity to transfer genetic material to other organisms as well as its capacity to being pathogenic for plants, animals or man. If the micro-organism carries relevant additional genetic elements, the **stability** of the encoded traits shall be indicated.
	B.2.9 Antibiotics and other anti-microbial agents	Information on the micro-organism's **resistance** or sensitivity to antibiotics or other anti-microbial agents must be provided, in particular the **stability** of the genes coding for antibiotic **resistance**, unless it can be justified that

Specific search term	Relevance for chapter*	Specific data requirement*
resistance		the micro-organism has no harmful effects on human or animal health, or that it can not transfer its **resistance** to antibiotics or other anti-microbial agents.

* The occurrence of the specific search term in the chapter heading and/ or in the specific data requirement is marked in bold

To cover the data requirements more comprehensively, several other specific search terms would have to be included in the literature search. Some suggestions are provided in Table B.2.10-4 .

Table B.2.10-4 Suggested specific search terms regarding the literature search for the data requirements concerning section B.2 "Biological properties of the micro-organism"

Chapter	Specific data requirement	Relevant search terms (suggestion)*
B.2.1.1 **Historical** background	The **historical** background of the micro-organism and its use (tests/research projects or **commercial use**) must be provided.	histor?, commercial use
B.2.1.2 **Origin** and natural **occurrence**	The geographical region and the place in the **ecosystem** (e.g. host plant, host animal, or soil from which the micro-organism was isolated) must be stated. The method of isolation of the micro-organism shall be reported. The natural **occurrence** of the micro-organism in the relevant **environment** shall be given if possible at strain level.	origin, occurrence, ecosystem, environment
B.2.2.2 **Mode of action**	The principal **mode of action** shall be indicated. In connection with the **mode of action** it shall also be stated if the micro-organism produces a toxin with a residual effect on the target organism. In that case, the **mode of action** of this toxin shall be described.	mode of action
	If relevant, information on the site of infection and **mode of entry** into the target organism and its susceptible stages shall be given. The results of any experimental studies must be reported.	mode of entry
	It shall be stated by which way an **uptake** of the micro-organism, or its **metabolites** (especially **toxins**) may occur (e.g. contact, stomach, inhalation). It must also be stated whether or not the micro-organism or its **metabolites** are **translocated** in plants and, where relevant, how this **translocation** takes place.	uptake, metabolite, toxin, translocat?

Chapter	Specific data requirement	Relevant search terms (suggestion)*
	In case of pathogenic effect on the target organism, infective dose (the dose needed to cause infection with the intended effect on a target species) and **transmissibility** (possibility of **spread** of the micro-organism in the target population, but also from one target species to another (target) species) after application under the proposed condition of use shall be indicated.	transmiss?, (vertical), (horizontal), spread
B.2.3 **Host specificity** range and effects on species other than the target harmful organism	Any available information on the effects on non-target organisms within the area to which the micro-organism may **spread** shall be given. The occurrence of non-target organisms being either closely related to the target species or being especially exposed shall be indicated.	host, specificity, spread
	Any experience of the **toxic** effect of the active substance or its **metabolic** products on humans or animals, of whether the organism is capable of colonising or invading humans or animals (including immunosuppressed individuals) and whether it is pathogenic shall be stated. Any experience of whether the active substance or its products may irritate skin, eyes or respiratory organs of humans or animals and whether it is allergenic in contact with skin or when inhaled shall be stated.	tox?, metabol?
B.2.4 **Development** stages/ **life cycle** of the micro-organism	Information on the **life cycle** of the micro-organism, described symbiosis, parasitism, competitors, predators, etc., including host organisms, as well as vectors for viruses, must be presented.	development, life cycle
	The generation time and the type of **reproduction** of the micro-organism must be stated.	reproduction, replication, proliferation
	Information on the occurrence of resting stages and their **survival** time, their **virulence** and **infection** potential must be provided.	survival, virulence, infection
	The potential of the micro-organism to produce **metabolites**, including **toxins** that are of concern for human health and/or the environment, in its different development stages after the release, must be indicated.	metabolite, toxin
B.2.5 **Infectiveness**, **dispersal** and **colonisation** ability	The **persistence** of the micro-organism and information on its life cycle under the typical environmental conditions of use must be indicated. In addition, any particular **sensitivity** of the micro-organism to certain compartments of the environment (e.g. **UV light**, **soil**, **water**) must be stated.	persistence, sensitivity, UV, light, soil, water, survival, infectiveness, reproduction, colonization, effectiveness, temperature, pH, humidity, virulence, metabolite, toxin
	The environmental requirements (**temperature**, **pH**, **humidity**, nutrition requirements, etc.) for **survival**, **reproduction**, **colonisation**, damage (including human tissues) and **effectiveness** of the micro-organism must be stated. The presence of specific **virulence** factors shall be indicated.	

Chapter		Specific data requirement	Relevant search terms (suggestion)*
		The **temperature** range at which the micro-organism grows must be determined, including minimum, maximum and optimum **temperatures**. This information is of particular value as a trigger for studies of effects on human health (Section 5).	
		The possible effect of factors such as **temperature**, **UV light**, **pH**, and the presence of certain substances on the stability of relevant **toxins** must also be stated.	
		Information on possible **dispersal** routes of the micro-organism (via air as dust particles or aerosols, with host organisms as vectors, etc.), under typical environmental conditions relevant to the use, must be provided.	dispersal
B.2.6	Relationships to known plant or animal or human **pathogens**	The possible existence of one or more species of the genus of the active and/or, where relevant, contaminating micro-organisms known to be **pathogenic** to humans, animals, crops or other non-target species and the type of **disease** caused by them shall be indicated. It shall be stated whether it is possible, and if so, by which means to clearly distinguish the active micro-organism from the **pathogenic** species.	pathogen?, disease
B.2.7	Genetic **stability** and factors affecting it	Where appropriate, information on genetic **stability** (e.g. mutation rate of traits related to the mode of action or uptake of exogenous genetic material) under the environmental conditions of proposed use must be provided.	stability
		Information must also be provided on the micro-organism's capacity to **transfer** genetic material to other organisms as well as its capacity to being pathogenic for plants, animals or man. If the micro-organism carries relevant additional **genetic elements**, the **stability** of the encoded traits shall be indicated.	horizontal transfer, genetic element, transposable element, transpos?
B.2.8	Information on the production of **metabolites** (especially **toxins**)	If other strains belonging to the same microbial species as the strain subject to the application are known to produce **metabolites** (especially **toxins**) with unacceptable effects on human health and/or the environment during or after application, the nature and structure of this substance, its presence inside or outside the cell and its stability, its mode of action (including external and internal factors of the micro-organism necessary to action) as well as its effect on humans, animals or other non-target species shall be provided.	metabolite, toxin
		The conditions under which the micro-organism produces the **metabolite(s)** (especially **toxin(s)**) must be described.	
		Any available information on the mechanism by which the micro-organisms regulate the production of the(se) **metabolite(s)** shall be provided.	

Chapter	Specific data requirement	Relevant search terms (suggestion)*
	Any available information on the influence of the produced **metabolites** on the micro-organism's mode of action shall be provided.	
B.2.9 **Antibiotics** and other **anti-microbial** agents	Many micro-organisms produce some **antibiotic** substances. Interference with the use of **antibiotics** in human or veterinary medicine must be avoided at any stage of the development of a microbial plant protection product.	antibiotic, antimicrobial, resistance
	Information on the micro-organism's **resistance** or **sensitivity** to **antibiotics** or other **anti-microbial** agents must be provided, in particular the stability of the genes coding for **antibiotic resistance**, unless it can be justified that the micro-organism has no harmful effects on human or animal health, or that it can not transfer its **resistance** to **antibiotics** or other **anti-microbial** agents.	

* Specific search terms used in the focused literature search by the notifier are underlined

B.2.10.3 References

KMA 2.1.1

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 2.1.1	OECD	2002	CONSENSUS DOCUMENT ON INFORMATION USED IN THE ASSESSMENT OF ENVIRONMENTAL APPLICATIONS INVOLVING BACULOVIRUS not available, not applicable ENV/JM/MONO, 1, 1-90 GLP/GEP: no Published: yes 3682775	no	no	not protected	-	Y KIIM 2.1

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 2.1.1	Tanada, Y., Kaya, H.K.	1993	DNA-VIRAL INFECTIONS: BACULOVIRIDAE not available, not applicable In: Insect Pathology. Academic Press (ed.), 171-244 GLP/GEP: no Published: yes 3682776	no	no	not protected	-	Y KIIM 2.1
KMA 2.1.1	Huber, J.	1990	HISTORY OF THE CPGV AS A BIOLOGICAL CONTROL AGENT - ITS LONG WAY TO A COMMERCIAL VIRAL PESTICIDE not available, not applicable not available GLP/GEP: no Published: yes 2019086	no	no	not protected		Y KIIM 2.1
KMA 2.1.1	Copping, L.G.	1998	THE BIOPESTICIDE MANUAL not available, not applicable British Crop Protecion Council, 60-61 GLP/GEP: no Published: yes 3682777	no	no	not protected	-	Y KIIM 2.1
KMA 2.1.1	Jones, K.A.	2000	BIOASSAYS OF ENTOMOPATHOGENIC VIRUSES not available, not applicable In: Navon, A., Ascher, K.R.S. (eds.)- Bioassays of entomopathogenic microbes and nematodes, 95-140 GLP/GEP: no Published: yes 3682778	no	no	not protected	-	Y KIIM 2.1

KMA 2.1.2

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 2.1.2	OECD	2002	CONSENSUS DOCUMENT ON INFORMATION USED IN THE ASSESSMENT OF ENVIRONMENTAL APPLICATIONS INVOLVING BACULOVIRUS not available, not applicable ENV/JM/MONO, 1, 1-90 GLP/GEP: no Published: yes **Submitted in: KMA 2.1.1** 2019088	no	no	not protected	-	Y KIIM 2.1
KMA 2.1.2	Krieg, A.	1976	GRANULOSIS AND NUCLEAR POLYHEDROSIS VIRUSES: SAFETY ASPECTS CONCERNING THEIR PRODUCTION AND APPLICATION not available, not applicable Z Angew Entomol, 82, 129-134 GLP/GEP: no Published: yes 3682706	no	no	not protected	-	Y KIIM 2.2
KMA 2.1.2/01	Kessler, P.	2010a	DECLARATION OF ORIGIN CPGV ISOLATE ABC-V15 (DSMZ GV-00013) Andermatt Biocontrol AG, CH, not stated Andermatt Biocontrol AG, Grossdietwil, Switzerland GLP/GEP: no Published: no 3306437	no	yes	New data for active ingredient, not previously submitted nor evaluated	ABA	N

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 2.1.2/02	Kessler, P.	2010b	DECLARATION OF ORIGIN CPGV ISOLATE ABC-V22 (DSMZ GV-00014) Andermatt Biocontrol AG, CH, not stated Andermatt Biocontrol AG, Grossdietwil, Switzerland GLP/GEP: no Published: no 3306438	no	yes	protected	ABA	N
KMA 2.1.2/03	Kessler, P.	2008	DECLARATION ON THE ORIGIN AND CHARACTERIZATION OF THE ACTIVE INGREDIENT OF MADEX PLUS Andermatt Biocontrol AG, CH, not applicable Andermatt Biocontrol AG, Grossdietwil, Switzerland GLP/GEP: no Published: no 3306439	no	yes	New data for active ingredient, not previously submitted nor evaluated	ABA	N
KMA 2.1.2/04	Jehle, J., Eberle, K.	2009a~~b~~	COMPARATIVE RESTRICTION ANALYSIS OF V15 Andermatt Biocontrol AG, CH, not stated DLR-Rheinpfalz, Neustadt, Germany GLP/GEP: no Published: no **Submitted in: KMA 1.3/04** 3306433	no	yes	protected	ABA	N
KMA 2.1.2/05	Jehle, J., Eberle, K.	2009b~~a~~	COMPARATIVE RESTRICTION AND PHYLOGENETIC ANALYSIS OF V22Andermatt Biocontrol AG, CH, not stated DLR-Rheinpfalz, Neustadt, Germany GLP/GEP: no Published: no **Submitted in: KMA 1.3/05** 3306434	no	yes	protected	ABA	N

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 2.1.2/06	Jehle, J.	2006c	COMPARATIVE RESTRICTION ANALYSIS OF CPGV (NEUSTADT MEXICAN ISOLATE) WITH CPGV (MADEX PLUS) Andermatt Biocontrol AG, CH, not applicable Dienstleistungszentrum Ländlicher Raum, Neustadt an der Weinstraße GLP/GEP: no Published: no **Submitted in: KMA 1.3/06** 3306435	no	yes	protected	ABA	N

KMA 2.2.1

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 2.2.1	Little, V.A.	1963	ORDERS TRICHOPTERA AND LEPIDOPTERA, FAMILY TORTRICIDAE (TORTRICIDS), THE CODLING MOTH not available, not applicable General and applied entomology, 2nd edition 324-326 GLP/GEP: no Published: yes 3682781	no	no	not protected	-	Y KIIM 2.3.1

KMA 2.2.2

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 2.2.2	Copping, L.G.	1998	THE BIOPESTICIDE MANUAL not available, not applicable British Crop Protecion Council, 60-61 GLP/GEP: no Published: yes **Submitted in: KMA 2.1.1** 3714761	no	no	not protected	-	Y KIIM 2.3.2
KMA 2.2.2	Tanada, Y., Leutenegger, R.	1968	HISTOPATHOLOGY OF A GRANULOSIS-VIRUS DISEASE OF THE CODLING MOTH, CARPOCAPSA POMONELLA not available, not applicable Journal of Invertebrate Pathology, 10, 39-47 GLP/GEP: no Published: yes 3714762	no	no	not protected	-	Y KIIM 2.3.2
KMA 2.2.2	Tanada, Y., Kaya, H.K.	1993	DNA-VIRAL INFECTIONS: BACULOVIRIDAE not available, not applicable In: Insect Pathology. Academic Press (ed.), 171-244 GLP/GEP: no Published: yes **Submitted in: KMA 2.1.1** 3714763	no	no	not protected	-	Y KIIM 2.3.2

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 2.2.2	Bilimoria, S. L.	1986	TAXONOMY AND IDENTIFICATION OF BACULOVIRUSES not available, not applicable The Biology of Baculoviruses, Biological Properties and Molecular Biology, Publisher: CRC Press, 1, 37-59 GLP/GEP: no Published: yes 3689461	no	no	not protected	-	Y KIIM 1.3.3
KMA 2.2.2	Steineke, S.B.	2004	POPULATIONSDYNAMIK DES CYDIA POMONELLA GRANULOVIRUS not available, not stated Dissertation zur Erlangung des Grades “Doktor der Naturwissenschaften” am Fachbereich Biologie der Johannes Gutenberg-Universität in Mainz, pp. 134 GLP/GEP: no Published: yes 2019090	no	no	not protected	-	Y KIIM 2.3.2
KMA 2.2.2	Steineke, S.B., Jehle, J.A.	2004	INVESTIGATING THE HORIZONTAL TRANSMISSION OF THE *CYDIA POMONELLA* GRANULOVIRUS (CPGV) IN A MODEL SYSTEM not available, not applicable Biological Control, 30, 538-545 GLP/GEP: no Published: yes 2019092	no	no	not protected	-	N

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 2.2.2	Kienzle, J., Schulz, C, Zebitz, C.P.W., Huber, J.	2003	PERSISTENCE OF THE BIOLOGICAL EFFECT OF CODLING MOTH GRANULOVIRUS IN THE ORCHARD -PRELIMINARY FIELD TRIALS not available, not applicable Insect Pathogens and Insect Parasitic Nematodes, IOBC wprs Bulletin, 26 (1), 245-248 GLP/GEP: no Published: yes 3682783	no	no	not protected	-	Y KIIM 2.3.2

KMA 2.3

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 2.3	OECD	2002	CONSENSUS DOCUMENT ON INFORMATION USED IN THE ASSESSMENT OF ENVIRONMENTAL APPLICATIONS INVOLVING BACULOVIRUS not available, not applicable ENV/JM/MONO, 1, 1-90 GLP/GEP: no Published: yes **Submitted in: KMA 2.1.1** 3682775	no	no	not protected	-	Y KIIM 2.3.2

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 2.3	Gröner, A.	1986	SPECIFICITY AND SAFETY OF BACULOVIRUSES not available, not applicable The Biology of Baculoviruses, Volume I, Biological Properties and Molecular Biologie, Chapter 9, 177-201 GLP/GEP: no Published: yes 3682784	no	no	not protected	-	Y KIIM 2.4
KMA 2.3	Fritsch, E., Huber, J., Backhaus, H.	1990	CPGV AS A TOOL IN THE RISK ASSESSMENT OF GENETICALLY ENGINEERED BACULOVIRUSES not available, not applicable Vth International Colloque on Invertebrate Pathology and Microbial Control, Adelaide, Australia, 439-443 GLP/GEP: no Published: yes 2390234	no	no	not protected	-	Y KIIM 2.4
KMA 2.3/01	Züger, M.	2011a	HOST RANGE CPGV ISOLATE ABC-V14 I 2011 Andermatt Biocontrol AG, CH, not stated Andermatt Biocontrol AG, Grossdietwil, Switzerland GLP/GEP: no Published: no 3714765	no	yes	protected	AND[10]	N

[10] Andermatt Biocontrol GmbH

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 2.3/02	Züger, M.	2011b	BIOASSAY WITH CPGV ISOLATE ABC V14 AGAINST CPGV-SENSITIVE AND CPGV-RESISTANT LARVAE OF CYDIA POMONELLA Andermatt Biocontrol AG, CH, not stated Andermatt Biocontrol AG, Grossdietwil, Switzerland GLP/GEP: no Published: no 3714766	no	yes	protected	AND	N
KMA 2.3/03	Züger, M	2017a	AW: HOST RANGE CPGV ISOLATE ABC-V45 I 2017 Andermatt Biocontrol AG, CH, not stated not available GLP/GEP: no Published: no 3714813	no	yes	protected	ABA	N
KMA 2.3/04	Züger, M	2017b	AW: BIOASSAY WITH CPGV ISOLATE ABC-V45 AGAINST GRAPHOLITA MOLESTA, CPGV SENSITIVE AND RESISTANT LARVAE OF CYDIA POMONELLA I 2017 Andermatt Biocontrol AG, CH, not stated not available GLP/GEP: no Published: no 3714814	no	yes	protected	ABA	N

KMA 2.4

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 2.4	Evans, H.F., Harrap, K.A.	1982	PERSISTENCE OF INSECT VIRUSES not available, not applicable Virus Persistence, Publisher: Cambridge University Press, 58-96 GLP/GEP: no Published: yes 3682785	no	no	not protected	-	Y KIIM 2.5
KMA 2.4	OECD	2002	CONSENSUS DOCUMENT ON INFORMATION USED IN THE ASSESSMENT OF ENVIRONMENTAL APPLICATIONS INVOLVING BACULOVIRUS not available, not applicable ENV/JM/MONO, 1, 1-90 GLP/GEP: no Published: yes **Submitted in: KMA 2.1.1** 2019094	no	no	not protected	-	Y KIIM 2.1
KMA 2.4	Copping, L.G.	1998	THE BIOPESTICIDE MANUAL not available, not applicable British Crop Protecion Council, 60-61 GLP/GEP: no Published: yes **Submitted in: KMA 2.1.1** 3682777	no	no	not protected	-	Y KIIM 2.5

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 2.4	Hess, R.T., Falcon, L.A.	1987	TEMPORAL EVENTS IN THE INVASION OF THE CODLING MOTH, CYDIA POMONELLA, BY A GRANULOSIS VIRUS: AN ELECTRON MICROSCOPE STUDY not available, not applicable Journal of Invertebrate Pathology, 50, 85-105 GLP/GEP: no Published: yes 2019102	no	no	not protected		Y KIIM 2.5
KMA 2.4	Bilimoria, S. L.	1986	TAXONOMY AND IDENTIFICATION OF BACULOVIRUSES not available, not applicable The Biology of Baculoviruses, Biological Properties and Molecular Biology, Publisher: CRC Press, 1, 37-59 GLP/GEP: no Published: yes **Submitted in: KMA 2.2.2** 3689461	no	no	not protected	-	Y KIIM 1.3.3
KMA 2.4	Jaques, R.A.	1977	STABILITY OF ENTOMOPATHOGENIC VIRUSES not available, not applicable Miscellaneous Publications of the Entomological Society of America, 10 (3), 99 - 116 GLP/GEP: no Published: yes 3728861	no	no	not protected	-	Y KIIM 2.5
KMA 2.4	Kool, M., Ahrens, C.H., Vlak, J.M., Rohrmann, G.F.	1995	REPLICATION OF BACULOVIRUS DNA not available, not applicable Journal of General Virology, 76, 2103-2118 GLP/GEP: no Published: yes 2019110	no	no	not protected		Y KIIM 2.5

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 2.4/01	Huh, N.E., Weaver, R.F.	1990	IDENTIFYING THE RNA POLYMERASES THAT SYNTHESIZE SPECIFIC TRANSCRIPTS OF THE AUTOGRAPHA CALIFORNICA NUCLEAR POLYHEDROSIS VIRUS not available, not applicable Journal of General Virology, 71, 195-201 GLP/GEP: no Published: yes 3714815	no	no	not protected	-	N

KMA 2.5

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 2.5	Jaques, R.A.	1977	STABILITY OF ENTOMOPATHOGENIC VIRUSES not available, not applicable Miscellaneous Publications of the Entomological Society of America, 10 (3), 99 - 116 GLP/GEP: no Published: yes **Submitted in: KMA 2.4** 3714768	no	no	not protected	-	Y KIIM 2.8

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 2.5	Huber, J.	1982	COMPARISON OF FIELD PERSISTENCE OF CPGV AND AONPV not available, not applicable C.E.C. meeting of experts on "Virus production and Specific Control Techniques in Orchards", Darmstadt, 2-3 December, 1982 GLP/GEP: no Published: yes 3682714	no	no	not protected	-	Y KIIM 2.8
KMA 2.5	Schmid, A.	1974	INVESTIGATIONS OF THE PERSISTANCE OF THE GRANULOSIS VIRUS OF THE LARCH BUD MOTH ZEIRAPHERA DINIANA (GN.) IN THE ENVIRONMENT AND THE PROTECTIVE ACTION OF SOME SUBSTANCES not available, not applicable Z Angew Entomol, 76, 31-49 GLP/GEP: no Published: yes 3682823	no	no	not protected	-	Y KIIM 2.8
KMA 2.5	Steineke, S.B.	2004	POPULATIONSDYNAMIK DES CYDIA POMONELLA GRANULOVIRUS not available, not stated Dissertation zur Erlangung des Grades “Doktor der Naturwissenschaften” am Fachbereich Biologie der Johannes Gutenberg-Universität in Mainz, pp. 134 GLP/GEP: no Published: yes **Submitted in: KMA 2.2.2** 2019090	no	no	not protected	-	Y KIIM 2.3.2

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 2.5	Krieg, A., Gröner, A., Huber, J., Zimmermann, G.	1981	INACTIVATION OF CERTAIN INSECT PATHOGENS BY ULTRAVIOLET RADIATION not available, not applicable Journal of Plant Diseases and Protection, 88 (1), 38-48 GLP/GEP: no Published: yes 3682824	no	no	not protected	-	Y KIIM 2.8
KMA 2.5	Kienzle, J., Schulz, C, Zebitz, C.P.W., Huber, J.	2003	PERSISTENCE OF THE BIOLOGICAL EFFECT OF CODLING MOTH GRANULOVIRUS IN THE ORCHARD -PRELIMINARY FIELD TRIALS not available, not applicable Insect Pathogens and Insect Parasitic Nematodes, IOBC wprs Bulletin, 26 (1), 245-248 GLP/GEP: no Published: yes **Submitted in: KMA 2.2.2** 3682888	no	no	not protected	-	Y KIIM 2.8
KMA 2.5	David, W.A.L., Gardiner, B.O.C.	1967	THE PERSISTENCE OF A GRANULOSIS VIRUS OF PIERIS BRASSICAE IN SOIL AND IN SAND not available, not applicable Journal of Invertebrate Pathology 9, 342-347 GLP/GEP: no Published: yes 3682713	no	no	not protected	-	Y KIIM 2.8
KMA 2.5	Huber, J.	2000	CERTIFICATE OF ANALYSIS Andermatt Biocontrol GmbH / Probis GmbH, not applicable Biol. Bundesanst. für Land- und Forstwirtschaft, Darmstadt GLP/GEP: no Published: no 3682728	no	no	not protected	PKA	Y KIIM 2.8

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 2.5	Gröner, A., Knauf, Reuß	1990	CYDIA POMONELLA GRANULOSUS VIRUS (CPGV) HOE 083311 SUMMARY ON CHEMICAL AND PHYSICAL DATA Andermatt Biocontrol GmbH / Probis GmbH, A55428 AgrEvo, Hoechst and Schering, Marburg, Germany GLP/GEP: no Published: no 3682712	no	no	not protected	PKA	Y KIIM 2.8
KMA 2.5	Tanada, Y., Omi, E.M.	1974	PERSISTENCE OF INSECT VIRUSES IN FIELD POPULATIONS OF ALFALFA INSECTS not available, not applicable Journal of Invertebrate Pathology 23, 360-365 GLP/GEP: no Published: yes 3682711	no	no	not protected	-	Y KIIM 2.8
KMA 2.5	Thomas, E.D., Reichelderfer, C.F., Heimpel, A.M.	1973	THE EFFECT OF SOIL PH ON THE PERSISTENCE OF CABBAGE LOOPER NUCLEAR POLYHEDROSIS VIRUS IN SOIL not available, not applicable Journal of invertebrate Pathology, 21, 21-25 GLP/GEP: no Published: yes 3682710	no	no	not protected	-	Y KIIM 2.8
KMA 2.5	Evans, H.F., Harrap, K.A.	1982	PERSISTENCE OF INSECT VIRUSES not available, not applicable Virus Persistence, Publisher: Cambridge University Press, 58-96 GLP/GEP: no Published: yes **Submitted in: KMA 2.4** 3714767	no	no	not protected	-	Y KIIM 2.8

KMA 2.6

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
KMA 2.6	OECD	2002	CONSENSUS DOCUMENT ON INFORMATION USED IN THE ASSESSMENT OF ENVIRONMENTAL APPLICATIONS INVOLVING BACULOVIRUS not available, not applicable ENV/JM/MONO, 1, 1-90 GLP/GEP: no Published: yes **Submitted in: KMA 2.1.1** 3728852	no	no	not protected	-	Y KIIM 2.7.1

KMA 2.7

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 2.7	Harvey, J.P., Volkman, L.E.	1983	BIOCHEMICAL AND BIOLOGICAL VARIATION OF CYDIA POMONELLA (CODLING MOTH) GRANULOSIS VIRUS not available, not applicable Virology 124, 21-34 GLP/GEP: no Published: yes 3714770	no	no	not protected	-	Y KIIM 2.10

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 2.7	Croizier, G.	1996	ANALYSE DES VIRUS DE LA GRANULOSE DE CARPOCAPSA POMONELLA CONTENUS DANS UN CULOT ISOLE PAR CENTRIFUGATION A 15000G DE CHAD FILTRE. ORIGINE NPP Arysta LifeScience S.A.S., URA n°2209 Institut National de la Recherche Agronomique, France GLP/GEP: no Published: no 2019116	no	no	not protected	ALS	Y KIIM 2.10
KMA 2.7	Biache, G.	1998	COMPARISON OF CPGV ISOLATE FROM CARPOVIRUSINE 2000 WITH DARMSTADT ISOLATE Arysta LifeScience S.A.S., not applicable Institut National de la Recherche Agronomique, France GLP/GEP: no Published: no 2019118	no	no	not protected	ALS	Y KIIM 1.4.3.1
KMA 2.7	Croizier, G.	2001	CARPOCAPSA POMONELLA GRANULOSIS VIRUS ANALYSIS. NPP 1996 STANDARD AN NPP 2001 STANDARD Arysta LifeScience S.A.S., EP01630 Institut National de la Recherche Agronomique, France GLP/GEP: no Published: no 2388141	no	no	not protected	ALS	Y KIIM 2.10

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 2.7	Jehle J.	2006	COMPARATIVE RESTRICTION ANALYSIS CPGV (NEUSTADT MEXICAN ISOLATE) WITH CPGV (MADEX MEXICAN ISOLATE) Andermatt Biocontrol GmbH / Probis GmbH, not applicable DLR-Rheinpfalz, Neustadt, Germany GLP/GEP: no Published: no 3431947	no	no	not protected	PKA	Y KIIM 1.3.3
KMA 2.7	Hughes, A.L., Friedman, R.	2003	GENOME-WIDE SURVEY FOR GENES HORIZONTALLY TRANSFERRED FROM CELLULAR ORGANISMS TO BACULOVIRUSES not available, not applicable Molecular Biology and Evolution, 20, 979–987 GLP/GEP: no Published: yes 2019126	no	no	not protected	-	Y KIIM 2.10
KMA 2.7	Herniou, E.A., Luque, T., Chen, X., Vlak, J.M., Winstanley, D., Cory, J.S. O'Reilly, D.R.	2001	USE OF WHOLE GENOME SEQUENCE DATA TO INFER BACULOVIRUS PHYLOGENY not available, not applicable Journal of Virology, 75, 8117-8126 GLP/GEP: no Published: yes 3714747	no	no	not protected	-	N
KMA 2.7	Jehle, J.A., Fritsch, E., Nickel, A., Huber, J., Backhaus, H.	1995	TCL4.7: A NOVEL LEPIDOPTERAN TRANSPOSON FOUND IN *CYDIA POMONELLA* GRANULOSIS VIRUS not available, not applicable Virology, 207, 369-379 GLP/GEP: no Published: yes	no	no	not protected	-	N

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 2.7	Jehle, J.A., Nickel, A., Vlak, J.M. Backhaus, H.	1998	HORIZONTAL ESCAPE OF THE NOVEL TC1-LIKE LEPIDOPTERAN TRANSPOSON TCP3.2 INTO *CYDIA POMONELLA* GRANULOVIRUS not available, not applicable Journal of Molecular Evolution 46, 215–224 GLP/GEP: no Published: yes	no	no	not protected	-	N
KMA 2.7	Arends, H.M., Winstanley, D., Jehle, J.A.	2005	VIRULENCE AND COMPETITIVENESS OF *CYDIA POMONELLA* GRANULOVIRUS MUTANTS: PARAMETERS THAT DO NOT MATCH not available, not applicable Journal of General Virology, 86, 2731–2738 GLP/GEP: no Published: yes	no	no	not protected	-	N
KMA 2.7	Jehle, J.	2007	COMMUNICATION JOHANNES JEHLE, DLR Arysta LifeScience S.A.S. not available GLP/GEP: no Published: no 1693368	no	no	not protected	ALS	Y KIIM 2.10
KMA 2.7/01	Anonymous	2016	LITERATURE REVIEW REPORT ON CYDIA POMONELLA GRANULOVIRUS - BIOLOGICAL PROPERTIES Arysta LifeScience S.A.S., not applicable Arysta Lifescience, France GLP/GEP: no Published: no 3306440	no	yes	protected	ALS	N

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 2.7/02	Gilbert, C., Peccoud, J., Chateigner, A., Moumen, B., Cordaux, R., Herniou, E.A	2016	CONTINUOUS INFLUX OF GENETIC MATERIAL FROM HOST TO VIRUS POPULATIONS not available, not applicable PLoS Genetics, 12, 1-21 GLP/GEP: no Published: yes 3306441	no	no	not protected	-	N
KMA 2.7/03	Gilbert, C., Chateigner, A., Ernenwein, L., Barbe, V., Bézier, A., Herniou, E. A., Cordaux, R.	2014	POPULATION GENOMICS SUPPORTS BACULOVIRUSES AS VECTORS OF HORIZONTAL TRANSFER OF INSECT TRANSPOSONS not available, not applicable Nature Communications, 5, 3348 GLP/GEP: no Published: yes 3714821	no	no	not protected	-	N
~~KMA 2.7/03~~	~~Gebhardt, M.M., Eberle, K.E., Radtke, P., Jehle, J.A.~~	~~2014~~	~~BACULOVIRUS RESISTANCE IN CODLING MOTH IS VIRUS ISOLATE-DEPENDENT AND THE CONSEQUENCE OF A MUTATION IN VIRAL GENE PE38~~ ~~not available, not applicable~~ ~~PNAS, 111, 15711-15716~~ ~~GLP/GEP: no~~ ~~Published: yes~~ ~~3306442~~	~~no~~	~~no~~	~~not protected~~	~~-~~	~~N~~
KMA 2.7/04	Chateigner, A., Bézier, A., Labrousse, C., Jiolle, D., Barbe, V., Herniou, E.A.	2015	ULTRA DEEP SEQUENCING OF A BACULOVIRUS POPULATION REVEALS WIDESPREAD GENOMIC VARIATIONS not available, not applicable Viruses, 7, 3625-3646 GLP/GEP: no Published: yes 3714818	no	no	not protected	-	N

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 2.7/05	Gueli Alletti, G., Sauer, A.J., Weihrauch, B., Fritsch, E., Undorf-Spahn, K., Wennmann, J.T., Jehle. J.A.	2017	USING NEXT GENERATION SEQUENCING TO IDENTIFY AND QUANTIFY THE GENETIC COMPOSITION OF RESISTANCE-BREAKING COMMERCIAL ISOLATES OF CYDIA POMONELLA GRANULOVIRUS not available, not applicable Viruses, 9, 1-16 GLP/GEP: no Published: yes 3714820	no	no	not protected	-	N