Pest Management Regulatory Agency
Agence de réglementation de la lutte antiparasitaire

Regulatory Note

REG2000-10

Virosoft CP4
Cydia pomonella granulosis virus

The naturally occurring microorganism *Cydia pomonella* granulosis virus and the formulated product Virosoft CP4 have been granted temporary registration under Section 17 of the Pest Control Products Regulations. Virosoft CP4 is a viral insecticide for the reduction of codling moth damage on apple trees. These products were reviewed jointly by the Pest Management Regulatory Agency and the U.S. Environmental Protection Agency under the North American Free Trade Agreement Technical Working Group on Pesticides Program for *Joint Review of Microbials and Semiochemicals.*

This regulatory note provides a summary of data reviewed and the rationale for the regulatory decision concerning these products.

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Foreword

Health Canada's Pest Management Regulatory Agency (PMRA) has issued a temporary registration for baculovirus *Cydia pomonella* granulosis virus and Virosoft CP4, developed by Bio Tepp Inc., a Canadian biopesticide company. The end use product contains a naturally occurring viral insecticide for the reduction of codling moth damage on apple trees. These products were reviewed jointly by the PMRA and the U.S. Environmental Protection Agency (EPA) under the North American Free Trade Agreement (NAFTA) Technical Working Group on Pesticides Program for *Joint Review of Microbials and Semiochemicals.*

Microbials are increasingly being investigated for use as alternatives to conventional pesticides. Virosoft CP4 is a biological insecticide containing the baculovirus *Cydia pomonella* granulosis virus (CpGV). Baculoviruses are arthropod-specific viruses that have been widely described and characterized in the scientific literature for close to 40 years. The CpGV contained in Virosoft CP4 has been isolated from Microbial Ecozone 4 of Canada, which encompasses the Great Lakes Region of both the U.S. and Canada, the Northeastern U.S., and the Canadian Maritimes. Baculoviruses have a long history of safe use, and it is expected that Virosoft CP4 will pose low potential risk to human health and the environment, compared with conventional pesticides.

BioTepp Inc. will be providing confirmatory information as a condition of this temporary registration. Following the review of this new data, the PMRA will publish a proposed regulatory decision document and request comments from interested parties before proceeding with a final regulatory decision.

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1.0 Product characterization and analysis

1.1 Physical and chemical properties of Virosoft CP4

The concentrated Virosoft CP4 product was described as having a pH of 6.5–7.5 and a viscosity similar to a 10% saline solution but with a slight increase due to fat residues originating from the fat bodies of *Cydia pomonella* larvae in which the *Cydia pomonella* granulosis virus (CpGV) active ingredient has been produced. The corrosion character of the end use product was also described as being equal to that of a 10% salt solution, whereas the corrosion character of the diluted product would be similar to that of water (but specifically to that of 0.007% saline).

The guarantee for Virosoft CP4 is described as 0.07% CpGV by weight or minimum of 4×10^{13} occlusion bodies (OBs)/L. The product specifications should typically include the minimum and maximum guarantee, in terms of OBs, an item that can be corrected as a condition of registration. Limited storage stability data indicated that when stored at ambient temperature (23 ±2EC) for up to six months, Virosoft CP4 maintained its original potency level for only three months. Therefore, the label is revised to indicate a three-month maximum, and data supporting a longer storage time may be provided after registration.

1.2 Details of uses

Virosoft CP4 is a naturally occurring biological insecticide containing the baculovirus CpGV. The strain of CpGV found in Virosoft CP4 is a wild type isolated from codling moth larvae (Isolate No. C-XIII-10). This strain is reported to be specific for the codling moth *Cydia pomonella*. The active ingredient, CpGV, has not been genetically modified or engineered through recombinant nucleic acid procedures.

The proposed use is for the reduction of codling moth damage on apple trees. Virus particles must be ingested by larvae to be effective. Applications should be timed so that early-instar larvae on the surface of the fruit come in contact with the virus before entering the fruit. This product is to be applied by ground application equipment only. The first application should be made at 210 degree-days (F) after biofix as determined by first consistent moth catch in pheromone traps. Diluted Virosoft CP4 should be applied before egg hatching, after which it should be applied another three times at two-week intervals, for a total of two applications per generation.

1.3 Biological properties

Baculoviruses are arthropod-specific viruses that have been widely described and characterized in the scientific literature for close to 40 years. The CpGV contained in Virosoft CP4 was isolated from Microbial Ecozone 4 of Canada, which encompasses the Great Lakes Region of both the U.S. and Canada, the Northeastern U.S., and the Canadian Maritimes. Baculoviruses have a long history of safe use that includes a

significant amount of human exposure to the viruses. The major concern with products such as Virosoft CP4 is not the viruses themselves but the potential unintentional microbes and/or irritants that may be in the ground insect–virus material used to make the product. Although the scientific literature suggests that this should not be a major concern, the battery of tests for health effects and quality control required by the PMRA are intended to further reduce the possibility of any adverse effects on human health.

Baculoviruses consist of a circular DNA genome surrounded by layers of protective protein molecules. Virus particles are embedded in a crystalline protein matrix called an occlusion body. These OBs are ingested when the insect larva feeds on contaminated food and dissolve in the alkaline environment of the insect midgut to release the virus particles. After penetrating the peritrophic membrane, the virus lipoprotein fuses with the plasma membrane of a gut wall cell and releases its nucleocapsid into the cytoplasm. The virus replicates within the nucleus of the host cell. Infected cells eventually rupture and release newly formed virus particles into the gut lumen where they attack other cells. The progressive increase in new virus particles and subsequent destruction of an increasing number of host gut cells eventually leads to death of the host. Virus particles are released from the decaying host into the environment where they can infect other larvae.

2.0 Analytical data and methodology

2.1 Analysis for the active ingredient/microbial pest control agent

Several techniques are used to identify and characterize baculoviruses. These include analysis of proteins by sodium dodecyl sulfate – polyacrylamide gel electrophoresis (SDS–PAGE), serology, restriction enzyme analysis of DNA, bioassays, and host range. These techniques are important tools to differentiate strains and isolates of viruses. Biotepp Inc. used standard molecular techniques to differentiate their CpGV C-VIII-10 isolate from a European CpGV (CpGV-M1) isolate previously described in the published literature. Based on the data and the literature references provided in the submission, the microscopic features and DNA restriction enzyme analysis should be sufficient to differentiate the Virosoft CP4 CpGV from other isolates.

2.2 Analysis for microbial contaminants

The criteria for determining the quality of the product, specifically bacterial contamination, include (*i*) a maximum of one contaminant bacterium per 1000 viruses (i.e., a virus to bacteria ratio of 1000:1 or less) and (*ii*) the use of microbe-specific selection media to identify any primary human pathogenic bacteria. The presence of any primary human pathogens, regardless of the virus to bacteria ratio, will result in destruction of the batch. In addition to these tests, an intraperitoneal (IP) injection test (in mice) will be performed on each production batch. The IP test is to assure that no primary human pathogens are present in the ground insect material that may be present in the final formulation. Because numerous literature reports indicate that the possibility of human pathogens being present in these insects is very small, this test should not result in

the loss of much, if any of the product. However, the microbe-specific media testing, along with the IP tests, should eliminate the possibility of primary human pathogens being present in the final Virosoft CP4 formulation.

2.3 Analysis of other unintentional ingredients

No known toxic or hazardous substances are expected to be present in the technical or end-use products. As the active ingredient is produced in vivo in insect larvae, other ingredients in the final preparation will likely include various insect parts, which may be potential irritants, as well as other cell debris such as fat material.

3.0 Human health and safety testing

3.1 Summary of toxicity and pathogenicity studies with Virosoft CP4

See Appendix I.

3.2 Exposure assessment

Baculoviruses are naturally occurring viruses that are widespread in the environment. The viruses have been described in the scientific literature for approximately 40 years, no adverse effects on humans due to exposure to these viruses have been reported. The toxicity tests conducted by the applicant indicate a low potential for adverse effects with direct exposure to Virosoft CP4 and the active ingredient CpGV through inhalation of spray mist or contact with the skin and eyes. Contact exposure to residues on treated plant surfaces are also not expected to produce adverse effects. Furthermore, because baculoviruses are naturally occurring organisms, many, if not all, individuals are likely to have been exposed previously. Because the amount of virus that will be applied is small, a significant increase in potential exposure is not likely. Any increase in virus titre is likely to be negligible at most and there should not be any increase in potential adverse effects on human health. In addition, no indoor residential, school, or daycare uses appear on the label, so the product will not be used in these areas and no additional nondietary exposure will occur. Even if a significant increase in exposure to the virus occurs, the toxicity studies submitted by the applicant and the extensive reports in the scientific literature on the safety of viruses suggest that there should not be any additional risk of adverse effects due to exposure to CpGV.

3.3 Food and feed residue studies

The acute oral toxicology data submitted by the applicant and the lack of production of known mammalian toxins, in addition to a long history of research, use, and safety testing of baculoviruses, suggest that there will not be any additional risk of adverse effects to humans or animals because of the use of Virosoft CP4 on food and feed crops for insect control. The CpGV virus is a naturally occurring organism to which some environmental and dietary exposure is likely to be common for most individuals. Application of the

product to reduce codling moth should result in a negligible, if any, increase in virus exposure. Given the lack of toxic or pathogenic effects seen in laboratory animals given high doses of CpGV and the likely natural background exposure to CpGV and similar baculoviruses, a method to detect residues of CpGV is not necessary. Therefore, an exemption from the requirement of a residue limit under the *Food and Drugs Act* and Regulations is proposed for the residues of CpGV in or on all raw agricultural commodities when used as a bioinsecticide for the control of *Cydia pomonella* (codling moth) on apples.

3.4 Overall human health summary

Under the PMRA–EPA Joint Review program, BioTepp, Inc. submitted the registration package to support registration of Virosoft CP4, which contains the baculovirus *Cydia pomonella* granulosis virus (CpGV) as the active ingredient. This was reviewed and determined to be acceptable. Although additional information and clarification are required as product characterization deficiencies were identified, the method of production of Virosoft CP4 should result in a product that is safe to use and consistent from batch to batch. Baculoviruses have a long history of safe use that includes a significant amount of human exposure to the viruses. The major concern with products such as Virosoft CP4 is not the viruses themselves but the potential unintentional microbes and/or irritants that may be contained in the ground insect–virus material used to manufacture the product. Although the scientific literature suggests that this should not be a major concern, the battery of tests for health effects and quality control required by the PMRA and EPA are intended to further reduce the possibility of any adverse human health effects. Results of oral dosing of mice revealed some minor clinical signs in both the test and control animals. Because both the test and control animals shared similar effects, it appears that these effects were not caused by the test substance itself. None of the six test animals died during the course of the study, and the LD_{50} for CpGV was greater than the maximum single dose of 1×10^{10} OBs/kg. In the acute pulmonary toxicity test, mice were administered intranasally with 1×10^{10} OBs/kg of CpGV. Exposure to the test substance caused no mortalities and no adverse clinical effects. In the acute IP toxicity test, mice were injected with 1×10^{10} OBs/kg of CpGV. No mortalities occurred in either male or female test animals, and no clinical signs were noted in the female mice. However, male mice injected with the test substance displayed small to moderate skin reactions (scabs) and other unusual signs in the scrotum and sacrum areas. Due to deficiencies noted in the study design, the results of the study are considered supplemental. The study can be upgraded to acceptable with submission of a future IP batch analysis that either does not show the significant clinical effects described or includes a pathogenicity study that reveals the cause of such effects. As a condition of registration, IP tests will be routinely required as part of the batch analysis conducted during product manufacture. Because of the questionable nature of the results of the present IP study, a final decision about the product will be made based on a new IP batch analysis or replacement study that includes pathogenicity testing. In the acute dermal toxicity and irritation test, the test organism produced slight to moderate skin irritation through the 72-hour observation period in one of three rabbits exposed to a single dose of

1×10^{10} OBs/kg of CpGV. No additional irritation scores were provided, but the clinical signs described appeared to be minor and most cleared before the end of the 14-day observation period. Overall, the test organism was found to be slightly irritating. Instillation of 0.1 mL of CpGV containing 1×10^{10} OBs per mL did not produce any apparent adverse effects in the eyes of three rabbits.

An exemption from the establishment of a maximum residue limit (MRL) is proposed for the residues of CpGV in or on all raw agricultural commodities when used as a bioinsecticide to control codling moth on apple trees. Based upon the low level of toxicity of this organism in the majority of Tier I toxicity studies, an exemption from an MRL is warranted.

The formulants used in Virosoft CP4 would not be expected to pose any human health and safety concerns.

4.0 Environmental toxicology

4.1 Summary of toxicity and pathogenicity of Virosoft CP4 to nontarget organisms

See Appendix II.

4.2 Overall ecological effects summary

The submission relied heavily on published information on other baculoviruses to address the potential for adverse effects on nontarget organisms. However, actual test data were provided on the toxicity and pathogenicity of Virosoft CP4 on a number of lepidopteran and beneficial arthropods, as well as on the freshwater daphnid and the earthworm.

The Baculoviridae family is widely regarded as comprising the largest and most widely studied group of viruses pathogenic to insects and other arthropods. Baculoviruses have been reported from invertebrates in the orders Lepidoptera, Thysanura, Homoptera, Coleoptera, Trichoptera, Diptera, and Hymenoptera, as well as from several crustaceans and arachnids. Over 500 species of the invertebrate order Lepidoptera are known to be hosts for baculoviruses. No member of the Baculoviridae family is known to infect vertebrates or plants. Cross-infectivity studies have shown that most baculoviruses have a narrow host range, never exceeding the order and usually not the family of the host from which the virus was originally isolated. Commonly, the host range is restricted to the genus of the competent host. Viruses from the genus *Granulosis virus* have only been isolated from arthropods and the host range of granulosis viruses (GVs) is generally narrower than that of nuclear polyhedroviruses.

Tests for nontarget arthropod host specificity used bioassays and phase contrast microscopy to detect viral polyhedra in either whole bodies or in isolated tissues and fat bodies. The following fourteen nontarget terrestrial arthropod species were tested with crude Virosoft CP4 preparations: six lepidopteran insect pests (four Noctuidae: cabbage

looper (*Trichoplusia ni*), true armyworm (*Pseudaletia unipuncta*), fall armyworm (*Spodoptera frugiperda*), bertha armyworm (*Mamestra configurata*), and two Tortricidae: obliquebanded leafroller (*Choristoneura rosaceana*) and spruce budworm (*Choristoneura fumiferana*)); the housefly (*Musca domestica*); the lesser migratory grasshopper (*Melanoplus sanguinipes*); the predatory wolf spider (*Lycosa* sp.); the Asian ladybeetle (*Harmonia axyridis*);the parasitoid wasp (*Ascogaster quadridentata*); the honeybee (*Apis mellifera*); the thatching ant (*Formica* sp.); and the two-spotted spider mite (*Tetranychus urticae*). Mortality and infectivity were not reported in five of the six lepidopteran insects, including the two tortricids related to the codling moth. However, mortality was noted in the cabbage looper, but no evidence was found of infectivity caused by Virosoft CP4. The only other nontarget insects adversely affected by Virosoft CP4 treatments were the housefly larvae, which produced fewer pupae and adults, and the braconid wasp, which produced fewer adults from virus-infected codling moth larvae. No GV particles were observed in specimens of either nontarget species examined by microscopy, which suggests that Virosoft CP4 was not the direct cause of these effects. For the housefly, it is possible that chemical properties such as pH or the presence of residual insect debris in the crude virus suspension may have affected development of housefly larvae. Alternatively, there is a possibility that an activated latent (occult) virus could have caused these effects as the crude virus suspension may have contained other insect viruses. For the braconid wasp, the reduced number of adults hatching from codling moth larvae exposed to both parasitoid and Virosoft CP4 was likely an indirect consequence of the virus killing the host larva before pupation of the parasitoid, not a direct infection of the parasitoid by Virosoft CP4. Based on these results, it is possible that parasitoid populations in apple orchards could be adversely affected by Virosoft CP4 applications because of direct competition for the same host. No other species tested with Virosoft CP4 demonstrated any adverse treatment-related effects. A number of experimental deficiencies were identified, but overall the study was sufficiently complete to be considered acceptable for assessing the potential risk of Virosoft CP4 to nontarget arthropod species.

The study results and weight of evidence in the published literature on host specificity of baculoviruses suggest that Virosoft CP4 has a narrow host range limited to codling moth. Given that baculoviruses are usually specific to the host (and sometimes to closely related species) from which they were isolated, Virosoft CP4 would likely have a minimum impact on nontarget terrestrial arthropod species, including the monarch butterfly. Although it is possible that repeated serial passage through a nontarget anthropod species could alter the host specificity of Virosoft CP4, this process is believed to occur naturally in the environment and under the conditions of use would contribute low concentrations of virus to the environment.

Only one study was submitted on the acute and reproductive effects of Virosoft CP4 in aquatic invertebrates. Treatment with Virosoft CP4 did not significantly increase mortality or inhibit reproduction in female *Daphnia magna*. No evidence of GV particles was found in either adults or neonates examined by microscopy, indicating that Virosoft CP4 was not likely capable of cross-infecting daphnids. Although there were a number of

experimental deficiencies that made it difficult to predict the effect of Virosoft CP4 on aquatic invertebrates in small aquatic systems, the known host range of Virosoft CP4 on terrestrial invertebrates and the published literature on the effects of other baculoviruses on aquatic invertebrates indicate that Virosoft CP4 is not likely to adversely affect aquatic invertebrates under the proposed use pattern.

No studies were submitted on the effects of Virosoft CP4 on nontarget fish species, but based on other studies with other baculoviruses in which no adverse effects have been observed, Virosoft CP4 would present a low environmental risk to fish when used according to the proposed label directions.

While no studies were submitted on the effects of Virosoft CP4 on birds and wild mammals, results of published studies on other baculoviruses suggest that Virosoft CP4 is unlikely to infect any vertebrate cells or to produce any adverse effects in birds or mammals. Mammalian toxicology data following various routes of administration have also shown no adverse effects from Virosoft CP4. However, birds will be a major method for short- and long-range dispersal of CpGV in the environment and wild mammals will be a method for short-range dispersal.

Only one study was submitted on the effects of Virosoft CP4 on terrestrial non-arthropod invertebrates. The study on the earthworm *Lumbricus terrestris* was unacceptable because of a number of experimental deficiencies. However, because of the usually limited specificity of baculoviruses to arthropod invertebrates and the weight of evidence in the published literature that addressed effects of other baculoviruses on non-arthropod invertebrates, it is highly unlikely that Virosoft CP4 would produce any adverse effects on non-arthropod invertebrates when used at the maximum proposed label rate.

No information was supplied on the effect of Virosoft CP4 on plants. No published reports were found that suggest plants or plant cell lines are susceptible to baculoviruses. Most evidence to support the applicant’s claim that Virosoft CP4 would have no effects on plants is circumstantial and unpublished, i.e., since baculoviruses have been experimentally and commercially applied to plants for many years and no case of phytotoxicity or phytopathogenicity has been recorded, baculoviruses are not considered phytotoxic or phytopathogenic. Based on this hypothesis, it was assumed highly unlikely that Virosoft CP4 would be phytotoxic or phytopathogenic.

4.3 Conclusions and risk mitigation

As a result of the conclusion that Virosoft CP4 would have minimal nontarget impacts, no additional studies or data are required to complete the environmental risk assessment of the product. The need for data on environmental fate or advanced ecotoxicological data (i.e., Tier II) was not triggered under current requirements for the proposed product since results of initial Tier I tests did not meet the criteria that would require additional testing. Regarding the aqueous spray mixtures specified on the label, it is considered that the formulants in the end-product, in themselves, would not pose an environmental risk when

used at the proposed concentrations and application rate on apple trees. Consequently, it is considered that Virosoft CP4 would pose little environmental risk when used in accordance with the label directions. Furthermore, no special precautionary or environmental hazard statements are required on the Virosoft CP4 label.

5.0 Value assessment

Virosoft CP4 is proposed for control of codling moth (*Cydia pomonella*) in commercial apple orchards. The proposed rate of application is 250 mL of formulated product (10^{13} virus particles per hectare). The initial application is to be made at 210 degree-days with repeated applications at intervals of two weeks. A total of four applications are proposed per season.

Results from laboratory bioassays show that Virosoft CP4 is effective in killing codling moth larvae. Bioassays in which larvae were immersed in prepared suspensions of virus for five minutes and then reared in cups containing an artificial diet generated LC_{50} values ranging from 1.33×10^5 to 3.47×10^5 OBs/mL. Laboratory tests in which codling moth larvae were exposed to apples treated with prepared suspensions of virus generated LC_{50} values of 1.99×10^5 to 3.28×10^7 OBs/mL.

Results from small-plot field trials conducted in Quebec in 1998 and 1999 suggest that Virosoft CP4 is effective in reducing codling moth damage to fruit. Although results were variable, the most significant effects with Virosoft CP4 were in terms of reduction in deep entry damage to fruit caused by third to fifth instar larvae. Virosoft CP4 did not appear to be as effective in reducing damage caused by first and second instar larvae ("stings"). However, the submitted studies do not allow for an assessment of the lowest effective rate of application or the optimum frequency or timing of application. While the submitted data are insufficient to draw definitive conclusions regarding the relative performance of Virosoft CP4 and commercial chemical insecticide treatments, the levels of fruit damage reported for the Virosoft CP4 treatments in the 1999 trials suggest that Virosoft CP4 may not be as effective as some commercial chemical insecticide treatments for codling moth. However, since Virosoft CP4 is expected to have minimal impact on beneficial predators and parasites found in apple orchards, this product may be useful in an integrated pest management program for apple production.

As to timing of application, the proposed use instructions state to apply after 210 degree-days and another three times in two-week intervals. This use pattern is supported by the submitted efficacy studies that were conducted in areas where two generations of codling moth occur per season. Fewer applications per season should be sufficient in areas with only one generation of codling moth occur per year (e.g., Georgian Bay area of Ontario, Nova Scotia).

6.0 Overall conclusions

The results of the acute toxicity studies submitted indicate that the active ingredient in Virosoft CP4, CpGV, was not toxic when administered orally or dermally at maximum hazard doses. No signs of toxicity were found when the active ingredient was administered to the lungs. Slight skin irritation existed in the rabbit, but signs of irritation were largely cleared after 14 days post exposure. No signs of any adverse effects were noted in the primary eye irritation study. No reports have been published of hypersensitivity related to exposure during product development or in experimental research trials with the product.

Although no clinical signs of toxicity were observed in female mice in the acute IP toxicity test, males displayed small to moderate scabs and other unusual signs in the scrotum and sacrum areas. These could be the result of aggressive cage behaviour rather than the result of exposure to the active ingredient, as these signs were noted on day one of dosing. No mortalities were reported in either female or male mice. The study was classified as supplemental because of deficiencies in the test design. Consequently additional intraperitoneal batch analysis data are required to demonstrate that the adverse effects observed in males was not a direct result of exposure to the active ingredient.

The need for data on the short-term toxicity and pathogenicity and on setting MRL of food crops was not triggered under current requirements for the proposed product since the organism is naturally occurring and results of initial Tier I toxicology tests did not meet the criteria that would require additional testing or establishment of an MRL. Exemption of an MRL for the microbial active ingredient is recommended.

The environmental effects studies submitted indicated that the active ingredient in Virosoft CP4, CpGV, is specific to the original codling moth host. Host-range testing on 14 terrestrial arthropod species showed that Virosoft CP4 was pathogenic and infective only to the intended target, i.e., codling moth, and was not able to infect larvae of six other insect species belonging to the order Lepidoptera, including two other species of the family Tortricidae (obliquebanded leafroller and spruce budworm). Although increased mortality was noted in the cabbage looper, no evidence was found of infectivity caused by Virosoft CP4. Increased mortality, but no infectivity, was also noted in treated housefly larvae and in the hatching of adult parasitoid wasps from virus-infected codling moth larvae. Housefly mortality was not attributed to Virosoft CP4 but to other properties of the crude virus preparation or to an activated latent (occult) virus. Lower hatching success of parasitoid wasps from virus-infected codling moth larvae was a consequence of the virus killing the codling moth larvae before pupation of the parasitoid. No mortality or infectivity was noted in six other nontarget arthropod species tested, including the beneficial honeybee and Asian ladybeetle. Submitted studies on an aquatic arthropod (freshwater daphnid) and non-arthropod invertebrate (earthworm) were of questionable quality, but the evidence in the published literature is sufficient to indicate that GV baculoviruses are not likely to produce adverse effects (mortality or infectivity) in either of these two groups of nontarget organisms. Published studies on other baculoviruses

have also shown a very low potential for adverse effects in wild mammals and birds. As well, many years of experimental and commercial applications of other baculoviruses on agricultural crops and forests have demonstrated that plants are not adversely affected by these viruses.

The need for data on environmental fate or advanced ecotoxicological data (i.e., Tier II) was not triggered under current requirements for the proposed product as results of initial Tier I tests did not meet the criteria that would require additional testing.

Virosoft CP4 was shown to be effective in reducing codling moth damage to fruit. Although results were variable, the most significant effects with Virosoft CP4 were in terms of reduction in deep entry damage to fruit caused by third to fifth instar larvae. Virosoft CP4 did not appear to be as effective in reducing damage caused by first and second instar larvae ("stings"). However, the submitted studies do not allow for an assessment of the lowest effective rate of application or the optimum frequency or timing of application. While the submitted data are insufficient to draw definitive conclusions regarding the relative performance of Virosoft CP4 and commercial chemical insecticide treatments, the levels of fruit damage reported for the Virosoft CP4 treatments in the 1999 trials suggest that Virosoft CP4 may not be as effective as some commercial chemical insecticide treatments for codling moth. However, since Virosoft CP4 is expected to have minimal impact on beneficial predators and parasites found in apple orchards, this product may be useful in an integrated pest management program for apple production.

Virosoft CP4 was considered acceptable for registration, subject to the provision of confirmatory information noted in section 7.0.

6.1 Virosoft CP4 product label

See Appendix III.

7.0 Regulatory decision

Cydia pomonella Granulosis Virus and Virosoft CP4 have been granted temporary registrations for use on apples, pursuant to Section 17 of the Pest Control Product Regulations, subject to the following studies:

- an additional analysis of the microbial pest control agent is required;
- a revised quality assurance method incorporating the IP batch analysis;
- one production batch analysis on the Virosoft CP4;
- an Intraperitoneal study conducted on the same production batch;

- an insect bioassay performed upon the same production batch; and
- additional confirmatory efficacy data for Virosoft CP4.

List of Abbreviations

bw	body weight
CpGV	*Cydia pomonella* granulosis virus
EC_{50}	effect concentration 50%
EEC	expected environmental concentration
GV	granulosis virus
IP	intraperitoneal
LC_{50}	lethal concentration 50%
LD_{50}	lethal dose 50%
LOEC	lowest observable effect concentration
LOEL	lowest observable effect level
MAS	maximum average score
MRL	maximum residue limit
NOAEL	no observed adverse effect level
NOEC	no observed effect concentrate
NOEL	no observed effect level
OB	occlusion bodies
PAGE	polyacrylamide gel electrophoresis
SDS	sodium dodecyl sulfate
U.S.	United States

Appendix I Summary of toxicity and pathogenicity studies with Virosoft CP4

Acute Study	Species / Strain and Doses	LD_{50}, NOEL / NOAEL and LOEL	Target Organ / Significant Effects / Comments
Oral	Mouse - CD-1, 3/sex, 1 x 10^{10} OBs[1] /kg bw	LD_{50} > 1 x 10^{10} OBs/kg bw	No mortalities and no clinical signs of toxicity caused by exposure to the test substance. **LOW TOXICITY**
Pulmonary	Mouse - CD-1, 5/sex, 1 x 10^{10} OBs/kg bw	LD_{50} > 1 x 10^{10} OBs/kg bw	No mortalities and no clinical signs of toxicity caused by exposure to the test substance. **LOW TOXICITY**
Injection	Intraperitoneal Injection Mouse - CD-1, 3/sex 1 x 10^{10} OBs/kg bw	LD_{50} > 1 x 10^{10} OBs/kg bw	No mortalities. No clinical signs of toxicity observed in females, but all males displayed small to moderate scabs and other unusual signs in the scrotum and sacrum areas including stained or thinning fur, slight protrusion of the penis, and reddening and swelling of the scrotum. These effects may not be the result of exposure to the test substance. The test mice were group housed and scabbing and other unusual signs were noted on Day 1, suggesting that the effects were not the result of dosing. Aggressive cage behavior between the subject male mice could also account for the observed effects. Infectivity/pathogenicity of test substance was not addressed. Study classified as supplemental, and can be upgraded to acceptable with submission of a future IP batch analysis which either does not show the same significant clinical effects, or one which includes a pathogenicity study that addresses the cause of such effects. **SUPPLEMENTAL**
Dermal Irritation / Dermal Toxicity	Rabbit - NZW, 3 females, 2.2 - 2.3 mL single dose (equal to 1 x 10^{10} OBs/kg bw)	LD_{50} > 1 x 10^{10} OBs/kg bw (LD_{50} > 2000 mg/kg bw) MAS[2] = 1.3/8 (72 h)	No mortalities or signs of systemic toxicity. One animal displayed slight to moderate skin effects that were still present after 72 h. Irritation mostly cleared after 14 days. **SLIGHTLY IRRITATING**
Eye Irritation	Rabbit - NZW, 3 females, 0.1 mL single dose (equal to 1 x 10^{9} OBs)	MAS < 15/110	No adverse effects noted at 1, 24, 48 and 72 h post-treatment. **MINIMALLY IRRITATING**

[1] OBs = Occlusion Bodies

[2] MAS = Maximum Average Score

Appendix II Summary Table of Toxicity and Infectivity Studies on 16 Non-Target Species with Virosoft CP4.

Arthropod	Percent Survival[a]			Microscopy[c]
	Virus	Control	Difference[d]	
Trichoplusia ni Cabbage Looper (Noctuidae)	89± 3 (750)	99 ± 1 (150)	-10(*)	-25
Pseudaletia unipuncta True Armyworm (Noctuidae)	97 ± 3 (750)	100 ± 0 (150)	-3	-25
Spodoptera frugiperda Fall Armyworm (Noctuidae)	97 ± 2 (750)	95 ± 4 (150)	2	-25
Mamestra configurata Bertha Armyworm (Noctuidae)	96 ± 1 (750)	94 ± 2 (150)	2	-25
Choristoneura fumiferana Spruce Budworm (Tortricidae)	93 ± 2 (750)	95 ± 6 (150)	-2	-25
Choristoneura rosaceana Obliquebanded Leafroller (Tortricidae)	98 ± 3 (750)	99 ± 1 (150)	-1	-25
Ascogaster quadridentata[b] (parasitoid)	4 (114)	28 (115)	-24(*)	-25
Ascogaster quadridentata[b] (parasitoid)	38 (60)	50 (60)	-12	-25
Lycosa sp. Wolf Spider (Araneae)	51 (37)	50 (38)	1	-23
Apis mellifera Honey Bee	69 (64)	58 (60)	9	-25
Tetranychus urticae Spider Mite (Acari)	62 (100)	60 (100)	2	-25
Formica sp. (Thatching Ant)	92 60)	92 (60)	0	-25
Harmonia axyridis Asian Ladybeetle (Coccinellidae)	77 (60)	78 (60)	-1	-12
Melanoplus sanguinipes Lesser Migratory Grasshopper	81 (150)	76 (150)	5	-25

Arthropod	Percent Survival[a]			Microscopy[c]
	Virus	Control	Difference[d]	
Musca domestica Housefly	40 (200)	59 (200)	-19(*)	-25
Daphnia magna[e] (Freshwater Daphnid)	16.7 (12) 33.2 ± 12.2	8.3 (12) 40.9 ±5.2	+8.4 -13.5[f]	-25
Lumbricus terrestris (Earthworm)	88(86)	85(84)	3	-25

[a] Results for the five lepidopteran species are means and standard deviations of three tests. Each test comprised five repeats of treatment virus and one control group. Percent survival values for grasshoppers are means and standard deviations of two tests. Results for the other organisms are percent values based on one test. The number of individuals test (n) is indicated as superscript. Survival of codling moth larvae exposed to Virosoft CP4 (target species positive control treatment) was 31.6 ± 18.9 percent (mean and standard deviation), based on 15 tests.

[b] *Ascogaster*: values represent percent parasitism. Top and bottom numbers are percent values of tests with high (1.0E+07 OBs per mL) and low (2.0E+06 OBs per mL) virus concentrations, respectively.

[c] Microscopy: absence of occlusion bodies in all specimens examined (-). The total number of specimens indicated as superscript in brackets.

[d] Column difference: percent survival of virus treatment was higher than control treatment (+); percent survival of virus treatment was lower than control treatment (-). Tests of independence were conducted for each non-target organism. Statistically significant differences between virus and control treatments are indicated as (*) for $P < 0.001$.

[e] Top values indicate percent of dead females and bottom values number of neonates produced per female.

[f] Difference in percent inhibition of reproduction; not statistically significant using a Student's t-test with $\alpha = 0.05$.

Appendix III Product Label for Virosoft CP4

DIRECTIONS FOR USE:

Virosoft CP4 is a biological insecticide containing *Cydia pomonella* granulovirus. Virus particles must be ingested by larvae to be effective. Applications should be timed so that early-instar larvae on the surface of the fruit come in contact with the virus before entering the fruit. This product is to be applied by ground application equipment only.

250 mL of Virosoft CP4 will treat one hectare (2.5 acres). Application should be made in sufficient volume of water to ensure thorough coverage of the trees and fruit. Make the first application at 210 degree-days (F) after biofix as determined by first consistent moth catch in pheromone traps. Apply diluted Virosoft CP[4] with jet or conventional sprayers prior to egg hatching and thereafter, another 3 times in 2 week intervals for a total of 2 applications per generation. It is recommended that this product be applied in the late afternoon or on a cloudy day to avoid direct exposure to sunlight.

PRECAUTIONS:

Keep away from children, food and feedstuffs. Hazards to humans and domestic animals. May cause sensitization. Avoid contact with skin, eyes and clothing.

Do not apply directly to water or to areas where surface water is present or to intertidal areas below the mean high water mark. Keep out of lakes, ponds, or streams. Do not contaminate water by cleaning equipment or disposing of equipment washwaters.

Do not enter or allow worker entry into treated areas during the Restricted Entry Interval (REI) of 4 hours. PPE required for early entry to treated areas that is permitted under the Worker Protection Standard and that involves contact with anything that has been treated, such as plants, soil or water, is: coveralls, waterproof gloves, shoes plus socks and protective eyewear.

Applicators and other handlers must wear:

Personal Protective Equipment
- Long-sleeved shirt and long pants
- Waterproof gloves
- Shoes and socks
- Protective eyewear, goggles, or protective face shield

Follow the manufacturer’s instructions for cleaning/maintaining PPE. If there are no such instructions furnished, wash PPE clothing detergent and hot water. Keep and wash PPE separately from other laundry.

User Safety Recommendations

User should: (1) Wash hands before eating, drinking, chewing gum, using tobacco, or using the toilet. (2) Remove clothing immediately if pesticide gets inside, then wash thoroughly and put on clean clothing. (3) Remove PPE immediately after handling this product. Wash the outside of the gloves before removing them. As soon as possible, wash thoroughly and change into clean clothing.

FIRST AID:

If in eyes: Hold eye open and rinse slowly and gently with water for 15-20 minutes. Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye. If irritation or sensitization occurs and persists, seek medical attention

If on skin: Remove contaminated clothing immediately and rinse skin with plenty of water for 15 - 20 minutes. If irritation or sensitization occurs and persists, seek medical attention.

Take container, label or product name and Pest Control Product Registration Number with you when seeking medical attention

STORAGE:

Store unused product in original container away from children and direct sunlight. Store refrigerated at 4EC. Keep open bottle refrigerated. Stored product has a viable shelf life of 3 months.

DISPOSAL:

1. Triple- or pressure-rinse the empty container. Add the rinsings to the spray mixture in the tank.

2. Follow provincial instruction for any required additional cleaning of the container prior to its disposal.

3. Make the empty container unsuitable for further use.

4. Dispose of the container in accordance with provincial requirements.

5. For information on disposal of unused, unwanted product, contact the manufacturer or the provincial regulatory agency. Contact the manufacturer and the provincial regulatory agency in case of a spill, and for clean-up of spills.

NOTICE TO USER:

This control product is to be used only in accordance with the directions on this label. It is an offense under the *Pest Control Products Act* to use a control product under unsafe conditions.

NOTICE TO BUYER:

Seller's guarantee shall be limited to the terms set out on the label and subject thereto, the buyer assumes the risk to persons or property arising from the use or handling of this product and accepts the product on that condition.