Evaluation Report ERC2013-01

Cydia pomonella Granulovirus strain M

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Overview

Registration Decision for *Cydia pomonella* Granulovirus Strain M

Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the *Pest Control Products Act* and Regulations, has granted conditional registration for the sale and use of CYD-X Technical and CYD-X, containing the biological active ingredient *Cydia pomonella* Granulovirus strain M, for control of codling moth in apples.

An evaluation of available scientific information found that, under the approved conditions of use, the products have value and do not present an unacceptable risk to human health or the environment.

Although the risks and value have been found acceptable when all risk-reduction measures are followed, the applicant must submit additional scientific information as a condition of registration.

This Overview describes the key points of the evaluation, while the Science Evaluation provides detailed technical information on the human health, environmental and value assessments of CYD-X Technical and CYD-X.

What Does Health Canada Consider When Making a Registration Decision?

The key objective of the *Pest Control Products Act* is to prevent unacceptable risks to people and the environment from the use of pest control products. Health or environmental risk is considered acceptable[1] if there is reasonable certainty that no harm to human health, future generations or the environment will result from use or exposure to the product under its proposed conditions of registration. The Act also requires that products have value[2] when used according to the label directions. Conditions of registration may include special precautionary measures on the product label to further reduce risk.

To reach its decisions, the PMRA applies modern, rigorous risk-assessment methods and policies. These methods consider the unique characteristics of sensitive subpopulations in humans (for example, children) as well as organisms in the environment (for example, those most sensitive to environmental contaminants). These methods and policies also consider the nature of the effects observed and the uncertainties when predicting the impact of pesticides. For more information on how the PMRA regulates pesticides, the assessment process and risk-reduction programs, please visit the Pesticides and Pest Management portion of Health Canada's website at healthcanada.gc.ca/pmra.

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1 "Acceptable risks" as defined by subsection 2(2) of the *Pest Control Products Act.*

2 "Value" as defined by subsection 2(1) of the *Pest Control Products Act*: "the product's actual or potential contribution to pest management, taking into account its conditions or proposed conditions of registration, and includes the product's *(a)* efficacy; *(b)* effect on host organisms in connection with which it is intended to be used; and *(c)* health, safety and environmental benefits and social and economic impact."

What Is *Cydia pomonella* Granulovirus Strain M?

Cydia pomonella Granulovirus strain M is a naturally occurring baculovirus that is used as a microbial pest control agent for the control of codling moth (*Cydia pomonella*) larvae on apple trees. CYD-X is a commercial class insecticide containing *Cydia pomonella* Granulovirus strain M as the active ingredient. CYD-X is specific to the larvae of the codling moth (*Cydia pomonella*). CYD-X must be ingested by larvae to be effective. After death, the larvae disintegrate, releasing new occlusion bodies which may infect other codling moth larvae upon ingestion. Exposed codling moth larvae die 3–7 days after ingestion of CYD-X, depending on dosage and ambient temperature. Codling moth death may occur more quickly at higher temperatures and higher dosages. Some damage to the fruit may occur before the larvae die.

Health Considerations

Can Approved Uses of *Cydia pomonella* Granulovirus Strain M Affect Human Health?

***Cydia pomonella* Granulovirus strain M is unlikely to affect your health when CYD-X is used according to the label directions.**

People can be exposed to *Cydia pomonella* Granulovirus strain M when handling and applying CYD-X and when consuming treated produce.

When assessing health risks, several key factors are considered such as:

- the microorganism's biological properties (for example, production of toxic byproducts);
- reports of any adverse incidents;
- its potential to cause disease or toxicity as determined in toxicological studies; and
- the levels to which people may be exposed relative to exposures already encountered in nature to other isolates of this microorganism.

Toxicological studies in laboratory animals describe potential health effects from large doses in order to identify any potential pathogenicity, infectivity and toxicity concerns. Because of the close relationships within the family Baculoviridae, results and findings from studies with various other baculoviruses are considered applicable to *Cydia pomonella* Granulovirus strain M and may be used for risk assessment purposes.

Studies in the published literature examining the effects of the exposure of various baculoviruses to laboratory animals yielded no signs of toxicity, disease or irritation. No member of this family of viruses is known to infect vertebrate animals. Furthermore, baculoviruses are highly host specific and have only been found in arthropods. The presence of insect debris in CYD-X, however, may cause irritation if inhaled or exposed to the skin or eye. Finally, baculoviruses are commonly found in nature at relatively high levels.

The use of CYD-X is not expected to significantly increase the baculovirus level in the environment. Baculoviruses have also been used for biological insect control for over 100 years. There have been no adverse effects noted as a result of either natural populations of baculoviruses or to applications of baculovirus-based pesticides.

As is the case with all microbial pest control agents, *Cydia pomonella* Granulovirus strain M contains substances that can cause allergic reactions in people who are repeatedly exposed to it at high concentrations. However, these reactions can be avoided if farm workers and applicators follow label recommendations to minimize or limit exposure to CYD-X.

Occupational Risks From Handling CYD-X

Occupational risks are not of concern when CYD-X is used according to label directions, which include protective measures.

Workers using CYD-X can come into direct contact with *Cydia pomonella* Granulovirus strain M (that is, through contact with skin or eyes, or by inhalation). Although the potential for toxicity is low in individuals exposed to *Cydia pomonella* Granulovirus strain M, the presence of insect debris in the end-use product may cause irritation if inhaled or exposed to the skin or eyes. Sensitization may also occur upon repeated exposure to high concentrations of the product. For this reason, users must wear a long-sleeved shirt, long pants, shoes plus socks, water-proof gloves, eye goggles and a dust/mist filtering respirator (MSH/NIOSH approval number prefix TC-21C) or a NIOSH approved respirator with any N-95, R-95, P-95 or HE filter for biological products while handling, mixing/loading or applying the product and during all clean-up/repair activities.

For bystanders, exposure is expected to be much less than that of workers involved in loading and application activities and is considered negligible. Therefore, health risks to bystanders are not of concern.

Residues in Water and Food

Dietary risks from food and water are not of concern.

As part of the assessment process prior to the registration of a pesticide, Health Canada must determine whether the consumption of the maximum amount of residues, that are expected to remain on food products when a pesticide is used according to label directions, will not be a concern to human health. This maximum amount of residues expected is then legally established as a maximum residue limit under the *Pest Control Products Act* for the purposes of the adulteration provision of the *Food and Drugs Act*. Health Canada sets science-based maximum residue limits to ensure the food Canadians eat is safe.

Although isolates of *Cydia pomonella* Granulovirus are common in nature, the residues of *Cydia pomonella* Granulovirus strain M remaining on produce from the use of CYD-X are expected to be higher than levels naturally occurring on fruit; however, studies in the published literature on other baculoviruses have demonstrated a lack of toxicity when laboratory animals were exposed

via the oral route. Similarly, no signs of infectivity were observed in tissue culture testing. Furthermore, the mode of action associated with baculoviruses is not dependent on toxin-production. Therefore, dietary risks are minimal to non-existent and the establishment of a maximum residue limit is not required for *Cydia pomonella* Granulovirus strain M.

The likelihood of residues contaminating drinking water supplies is minimal. Consequently, dietary risks are also minimal.

Environmental Considerations

What Happens When *Cydia pomonella* Granulovirus strain M Is Introduced Into the Environment?

Environmental risks are not of concern.

Cydia pomonella Granulovirus strain M is a natural baculovirus isolate that infects and kills the larval life stage of codling moth insects. Baculoviruses are generally specific to certain insect species. No member of the Baculoviridae family is known to infect vertebrates or plants. Granuloviruses have only been reported from members of the order Lepidoptera (moths and butterflies). Infectivity of Granuloviruses is limited to insect species within the same family as the host from which it was originally isolated (codling moth; *Cydia pomonella*). In the case of *Cydia pomonella* Granulovirus strain M, infectivity is limited to the family Tortricidae. Effects on even more distantly-related non-target organisms are, therefore, not expected.

Furthermore, baculoviruses are ubiquitous in the environment. The use of CYD-X to control codling moth in apple orchards is not expected to significantly increase the baculovirus load in the environment beyond naturally-occurring levels. Baculoviruses, including other strains of *Cydia pomonella* Granulovirus, have been extensively used as biological control agents. No reports of adverse effects have been noted on non-target organisms due to either natural populations of baculoviruses or to applications of baculovirus-based pesticide products.

Value Considerations

What Is the Value of CYD-X?

CYD-X has value in controlling codling moth, which is a major pest of apples; it can be used by organic growers and in integrated pest management programs.

The crop and pest combination of apples and codling moth has been identified in the Grower Priority Database as a high priority and is present on the labels of three active ingredients (azinphos-methyl, diazinon and endosulfan) which are being phased out for use in Canada. *Cydia pomonella* Granulovirus can be used by organic growers and is another tool that be utilized in codling moth integrated pest management programs.

Measures to Minimize Risk

Labels of registered pesticide products include specific instructions for use. Directions include risk-reduction measures to protect human and environmental health. These directions must be followed by law.

The key risk-reduction measures on the label of CYD-X to address the potential risks identified in this assessment are as follows.

Key Risk-Reduction Measures

Human Health

Statements warning users that the product is a dermal and eye irritant, as well as a potential sensitizer, are required on the label.

To minimize exposure to mists generated while handling, mixing/loading or applying the product and during all clean-up/repair activities, users must wear a long-sleeved shirt, long pants, shoes plus socks, water-proof gloves, eye goggles and a dust/mist filtering respirator (MSH/NIOSH approval number prefix TC-21C) or a NIOSH approved respirator with any N-95, R-95, P-95 or HE filter for biological products.

Environment

As a general precaution, to reduce runoff, users must not apply the product to aquatic systems and contamination of irrigation or drinking water supplies and aquatic habitats is prohibited. Furthermore, users are directed to not apply the product by air and to follow application instructions to minimize spray drift. Standard disposal statements for unused or unwanted product and the product container also apply.

What Additional Scientific Information Is Being Requested?

Although the risks and value have been found acceptable when all risk-reduction measures are followed, the applicant must submit additional scientific information as a condition of registration. More details are presented in the Science Evaluation of this Evaluation Report or in the Section 12 Notice associated with these conditional registrations. The applicant must submit the following information by 1 September 2013:

Product Characterization and Analysis

- Additional microbial contaminant analysis data on a minimum of four batches of CYD-X.
- Protocols for microbial contaminant analysis testing of the indicator micro-organisms.
- Stability data under storage conditions consistent with label directions on a minimum of three additional batches of CYD-X.

Other Information

As these conditional registrations relate to a decision on which the public must be consulted, [3] the PMRA will publish a consultation document when there is a proposed decision on applications to convert the conditional registrations to full registrations or on applications to renew the conditional registrations, whichever occurs first.

The test data cited in this Evaluation Report (that is, the test data relevant in supporting the registration decision) will be made available for public inspection when the decision is made to convert the conditional registrations to full registrations or to renew the conditional registrations (following public consultation). If more information is required, please contact the PMRA's Pest Management Information Service by phone (1-800-267-6315) or by e-mail (pmra.infoserv@hc-sc.gc.ca).

[3] As per subsection 28(1) of the *Pest Control Products Act.*

Science Evaluation

Cydia pomonella Granulovirus strain M

1.0 The Active Ingredient, Its Properties and Uses

1.1 Identity of the Active Ingredient

Active microorganism	*Cydia pomonella* Granulovirus strain M
Function	Viral insecticide
Binomial name	*Cydia pomonella* Granulovirus strain M
Taxonomic designation	
Family	Baculoviridae
Genus	Granulovirus
Species	*Cydia pomonella* Granulovirus
Strain	Strain M
Patent Status information	n/a
Purity of active ingredient	$>6 \times 10^{13}$ occlusion bodies (OB)/L (equivalent to 0.12% w/w)
Identity of relevant impurities of toxicological, environmental and/or significance	CYD-X Technical does not contain any impurities or microcontaminants known to be Toxic Substances Management Policy (TSMP) Track 1 substances. The product must meet microbiological contaminants release standards and no mammalian toxins are known to be produced by the microbial pest control agent, *Cydia pomonella* Granulovirus strain M.

1.2 Physical and Chemical Properties of the Active Ingredients and End-use Product

Technical Product—CYD-X Technical

Property	Result
Colour	Dark gray brown
Physical state	Liquid
pH	6.3–6.5

Property	Result
Viscosity	46 mPa/s at 20°C
Miscibility	miscible
Specific gravity	1.167 g/mL
Storage stability	One year when stored at 4°C

End-use Product—CYD-X

Property	Value
Colour	Dark gray brown
Physical state	Liquid
Guarantee	$>3 \times 10^{13}$ occlusion bodies (OB)/L (equivalent to 0.06% w/w)
pH	6.3–6.5
Viscosity	46 mPa/s at 20°C
Miscibility	miscible
Specific gravity	1.167 g/mL
Storage stability	One year when stored at 4°C

1.3 Directions for Use

CYD-X is for use in apples to control codling moth larvae. This product is to be applied at a rate of 250 mL per hectare in sufficient water for thorough coverage of the tree canopy. Application timing is against small larvae just after egg hatch and prior to entering the fruit; early in the spring for the first generation and later in the summer for the second generation.

1.4 Mode of Action

CYD-X must be ingested by codling moth larvae to become infected with the virus. Upon ingestion, the viral occlusion bodies (OBs) dissolve in the larvae midgut and release infectious virions. The virions then enter the cell that line the digestive tract and replicate in the nuclei of these cells. The resulting replicated virions rapidly spread the infection to the other organs within the larva. Within a few days after ingestion of CYD-X, the infected larva stops feeding, becomes sluggish and discoloured and eventually dies from a massive viral infection. After death, the larvae disintegrates, releasing new OBs which may infect other codling moth larvae upon ingestion. Exposed codling moth larvae die within 3 to 7 days after ingestion of CYD-X, depending on dosage and ambient temperature. Codling moth death may occur more quickly at higher temperatures and higher dosages.

2.0 Methods of Analysis

2.1 Method for Identification of the Microorganism

Restriction endonuclease (REN) analysis is used to confirm the identity of the CYD-X isolate as well as to distinguish it from other closely related Granuloviruses or *Cydia pomonella* Granulovirus (CpGV) isolates. Separate REN analysis comparisons of the CYD-X isolate against the Neustadt (Mexican) CpGV isolate and the Neustadt isolate against the Virosoft CpGV isolate were submitted. In order to confirm that REN analysis is capable of distinguishing CpGV strain M from other closely related CpGV isolates, the registrant has committed to conduct a side-by-side REN analysis comparing the CYD-X CpGV, the Mexican CpGV and the Virosoft CpGV isolates.

2.2 Method for Establishment of Purity of Seed Stock

The origin of supply of the Master Seed Bank (MSB) for the CYD-X isolate is Andermatt Biocontrol AG. After identification of the CYD-X isolate, Andermatt Biocontrol AG produces a certain quantity (for example, 50L) of the isolate as the MSB stock, which is kept at -20^{o}C and used for comparison studies such as bioassays and REN analysis as well as for production of the working seed bank (WSB) stock. The MSB is expected to be sufficient in quantity for at least 50 years of production. The quality of the MSB is initially tested in a bioassay against the original isolate. Bioassays are also used to test the WSB and all production batches against the MSB.

2.3 Method to Define the Content of the Microorganism in the Manufactured Material Used for the Production of Formulated Products

The guarantee (expressed as OBs/mL) is determined by bioassay against a reference standard with known virus concentration (as determined by hemacytometer count). The OBs/mL count is re-interpreted as a % weight of active ingredient by taking into account the theoretical mass of a Granulovirus and the density.

2.4 Method to Determine and Quantify Residues (Viable or Non-viable) of the Active Microorganism and Relevant Metabolites

REN analysis is used as an appropriate method to distinguish the residues of CpGV strain M from other isolates of this virus on food crops.

2.5 Method for Determination of Relevant Impurities in the Manufactured Material

The quality control procedures used to limit contaminating microorganisms during the manufacture of CYD-X Technical and CYD-X are acceptable. These procedures include contamination checks at the end of the manufacturing process. Certis USA is required to submit contaminant analysis data for an additional four batches of CYD-X to verify that the end-use product meets accepted microbial contamination release standards established by the PMRA for

baculovirus-based pesticides. Production batches that do not satisfy the contaminant release standards are to be discarded.

Furthermore, protocols are also required for screening and analysis of additional indicator microorganisms as identified by the PMRA for baculovirus-based pesticides.

2.6 Method to Determine Storage Stability, Shelf-life of the Microorganism

Storage stability testing was conducted on two batches of CYD-X. The first batch was stored at 2°C and the second batch was stored at 25°C. The data were sufficient to demonstrate that CYD-X can be stored at these temperatures for up to one year. The labels for CYD-X Technical and CYD-X specify storage conditions of up to one year from the date of manufacture at 4°C. To confirm the acceptability of the storage conditions indicated on the label, additional storage stability data on three batches of CYD-X are required as a condition of registration.

3.0 Impact on Human and Animal Health

3.1 Toxicity and Infectivity Summary

The PMRA conducted a detailed review of the toxicological database for CpGV strain M, the active ingredient in CYD-X Technical and CYD-X. Requests to waive actual testing with the microbial pest control agent (MPCA) were deemed acceptable to address acute oral infectivity and toxicity, acute pulmonary infectivity and toxicity, intravenous infectivity, acute dermal toxicity and irritation, eye irritation and dermal sensitization. A tissue culture study was submitted. The database is considered sufficient to characterize the toxicity and infectivity of this pest control agent and product.

Waiver rationales were based on studies conducted with various other baculoviruses including both nucleopolyhedroviruses (NPVs) and Granuloviruses. Because of the close relationships within the family *Baculoviridae*, results from studies with NPVs and other Granuloviruses are considered applicable to CpGV strain M and may be used for risk assessment purposes.

In a feeding trial with the NPV and Granulovirus isolated from the larvae of *Mamestra brassicae* (cabbage moth), *Mamestra brassicae* nucleopolyhedrovirus (MbNPV), and *Laspeyresia pomonella* (codling moth), CmGV, respectively, mice were fed bread drenched with the virus suspension at a total dose of 3×10^9 MbNPV polyhedra/animal or 5×10^{11} CmGV capsules/animal (all of the bread was eaten). The total dose was administered to mice as either a single-dose or as 34 partial doses over a period of 99 days. Guinea pigs were also administered a total of 5×10^{11} MbNPV polyhedra/animal by gavage over a period of 99 days and 34 partial doses. No adverse effects were noted in either the single- or multiple-dose tests. Feeding continued with continuous appetite, weight gains were steady and the behaviour of the test animals did not differ from that of untreated control animals.

In another study, codling moth Granulovirus (CmGV) was administered to Chinese hamsters and NMRI mice via feed. NMRI mice (4/sex) and Chinese hamsters (7 males) were dosed with 5.2×10^9 CmGV capsules/animal/day over a period of 97 days and 1.7×10^{10} CmGV

capsules/animal/day over a period of 90 days, respectively. In addition, Chinese hamsters (8 males; 3 females) were also subjected to a single dose of 1.5×10^{12} CmGV capsules/animal. None of the animals died during the course of the study. In all experiments, the animals were examined daily for signs of health disturbances and abnormal behaviour. Deviations from the controls were not seen in any of the treatment groups. Food uptake and body weight were normal. Necropsies of the animals in the long-term studies revealed no morphological changes in the gastrointestinal tract or in the major organs.

The published scientific literature also included a report of an inhalation study in which two guinea pigs per group were exposed for five minutes to an aerosol produced by fine spraying of 3 mL of an aqueous suspension containing either 1×10^{10} polyhedral inclusion bodies (PIBs)/mL of MbNPV or 2×10^{10} granules/mL of CpGV in a 30 L exposure chamber. A control group was exposed to a water aerosol. During the 21-day observation period, no adverse effects on general health conditions were found in the test animals compared to the control animals. Food consumption, body weight gain, body temperature and behaviour remained normal. Necropsies including histological examinations revealed no signs of irritation in the lungs and respiratory passages. There were no antibodies and no visible alterations in the blood serum electrophoretogram (detects possible changes in ratios between serum albumin and the globulins). Meaningful conclusions could not be drawn from this study due to the short exposure period and the limited number of test animals.

A number of other pulmonary toxicity studies were found in the published scientific literature. Eighteen Sprague-Dawley rats were exposed for one hour to a concentrated dust of *Lymantria dispar* nucleopolyhedrovirus (virus concentration not provided), with a flow rate of 12 L/min inside an inhalation chamber. No signs of toxicity or abnormal behaviour were noted in any test animal during or following exposure. No animals died and no treatment-related abnormalities were recorded at necropsy (Lewis and Podgwaite, 1981).

Rats exposed to 3.6×10^{7} PIB of *Orygia pseudotsugata* nucleopolyhedrovirus (OgNPV)/kg bw via the inhalation route did not result in any deaths or signs of systemic toxicity or pathogenicity. Gross pathology examinations revealed no treatment-related abnormalities.

In a non-human primate study, two male and two female rhesus monkeys were exposed to an aerosol spray of a *Helcoverpa zea* NPV (HzNPV) suspension (1.2×10^{8} PIB) that was directed into the mouth. In addition, two drops of the suspension were instilled in each nostril. General health, body weight gains and body temperature of treated monkeys were similar to those of untreated controls. Hematology and blood chemistry did not detect any findings that might be attributed to treatment. Histopathology performed either on day 33 after dosing or after 26 weeks failed to elucidate treatment-related effects.

A summary of a study in which four NPVs and a Granulovirus were injected into mice or guinea pigs was submitted for review. For each virus, test animal, route of administration (intraperitoneal, intravenous, or subcutaneous), and virus form (granulin, polyhedron, or freed virions) combination, between 20 and 30 animals were tested. An equal number of animals served as controls in the intraperitoneal and intravenous tests. The dose administered was between 1×10^{8}–3.5×10^{10} particles (OBs or virions) per animal. Mortalities were observed in

the test groups but were comparable or less frequent than in the control groups. There was no evidence of toxicity or pathogenicity in the tests.

Several other studies were found in the published scientific literature in which baculoviruses were injected into animals. Mice were administered a single intraperitoneal injection of 1.6×10^6 PIBs/kg bw or 1.6×10^7 PIBs/kg bw of OgNPV. Aside from one death that occurred at the highest dose level on the day of injection that was not attributed to the treatment, there were no signs of systemic toxicity or pathogenicity.

In an intravenous injection study, ten female Sprague-Dawley rats were dosed with 12×10^8 PIB of HzNPV. The animals were observed for three weeks. There were no mortalities and no significant differences were observed, with regard to body weight gain, in comparison to the saline-treated control group. There were no pathological changes at necropsy.

The dermal irritation potential of MbNPV and *Laspeyresia pomonella* Granulovirus (LpGV) was tested in guinea pigs. Four dermal sites on each animal were abraded and rubbed with 0.05 mL of either MbNPV or LpGV (in physiological saline solution) corresponding to doses of 5×10^7 PIBs/animal/site or 1×10^9 granulin capsules/animal/site, respectively. Negative control sites were dosed with physiological saline solution. The test material was left in contact with the skin for 72 hours. No dermal reactions were observed. No antibodies were found in the serum and no changes in blood protein profile were noted 14 days after exposure.

The eye irritation potential of MbNPV and LpGV was tested in guinea pigs. A total volume of 0.05 mL of either MbNPV or LpGV (in physiological saline solution) corresponding to doses of 5×10^7 PIBs/animal/site or 1×10^9 granulin capsules/animal/site, respectively, were applied to the corneas. Negative control corneas were dosed with physiological saline solution. The test material was left in contact with the eye for 72 hours. No ocular reactions were observed.

No sensitizing properties were detected with a commercial preparation of HzNPV in guinea pigs following inahalative or intradermal exposure. The material was applied by inhalation in the form of PIBs and free virus rods at a daily dose of 3×10^{10} PIB/animal for five days per week for three consecutive weeks. Another group of guinea pigs received an intradermal induction with 1.5×10^{10} PIB/mL corn oil applied to the scarified skin for five days and challenge was after 19 days by intradermal injection of 1.2×10^8 PIB. No signs of sensitization were observed by either administration route.

In a tissue culture study, the mammalian cell lines WI-38 and Detroit 551were exposed to CpGV strain M in the form of liberated granulin-derived virus particles. Cells were inoculated at a multiplicity of infection equivalent to 58–62 LD90s/cell (the LD90 was determined by bioassay on codling moth larvae). Any unabsorbed virus was removed and the cells were rinsed and incubated at 37°C or at 4°C for seven days. As mammalian cells stored at 4°C are metabolically inactive and cannot support virus replication, the cells stored at 4°C served as controls to account for any residual virus remaining in the flasks after rinsing. Negative controls consisting of uninoculated cells were also maintained. The cells were then harvested, fractionated and subjected to bioassay against codling moth larvae. The bioassay results indicated that there was no increase in the quantity of virus above the residual inoculum level. Furthermore, the

inoculated cells did not exhibit any signs of virus replication or cytopathic effects. Therefore, CpGV strain M did not appear to replicate in mammalian cells.

Numerous studies have been conducted to assess the ability of baculoviruses to enter, infect or persist in mammalian cells with the general consensus being that baculoviruses can enter such cells, but that there are no cytopathic effects even at very high multiplicities of infection.

Inoculation of HeLa, W1-38 (human diploid embryonic lung cells), HEK and AGMK (African green monkey kidney) cells with HzNPV did not result in any cytopathic effects. A similar study using PHA (primary human amnion), HF (foreskin), embryo (EM), WI38 (lung) and LEU (leukocytes) cell lines and HzNPV (both PIBs and budded virions) did not indicate any cytopathic effects or adverse effect on cell viability.

A more extensive study was conducted in which 35 cell lines (23 of human origin and 12 of non-human mammalian origin) were assessed. Each cell line was incubated at two different temperatures (28°C and 37°C) and the cells were observed at four time points ranging from 16 to 168 hours post-inoculation and virus entry was monitored by a peroxidase-antiperoxidase assay. *Autographa californica* (Ac) NPV preparations derived from polyhedra, hemolymph, cell culture medium and infected cells were used as inocula. No evidence of replication was observed in any of the 35 cell lines tested, although virus uptake appeared to be quite common. Subsequent studies confirm the finding that *Autographa californica* nucleopolyhedrovirus is capable of entering mammalian cells but virus particles are found only in phagocytic vacuoles or in the cytoplasm, not in the nucleus, and that there are no cytopathic effects or increase in chromosomal abnormalities.

Moreover, baculoviruses are known to have a limited host range. Baculoviruses have only been found in arthropods. No member of this family is known to infect vertebrates. Granuloviruses are reported only from lepidopteran hosts. Infectivity of Granuloviruses is limited to insect species within the same family as the host from which the Granulovirus was originally isolated. In the case of CpGV, infectivity is limited to very few hosts of the Tortricidae family only.

The presence of the crystalline protein matrix of occluded virions presents one barrier to the ability of baculoviruses to infect nontarget organisms. In order to release the virions to initiate infection, alkaline conditions are required to dissolve the protein matrix. Organisms that lack such alkaline conditions in their digestive tract or other potential points of entry should not be susceptible to infection by the occluded form of the virus (as is the case with baculovirus-based biopesticide products).

In the event that the organism is infected with an “active” form of the virus (for example, budded virus or alkaline-liberated virus), restrictions at the molecular level within cells would block viral replication. Infection is dependent on the promoter of the baculovirus that is active only in Lepidoptera. Host specificity has been demonstrated in vitro in numerous mammalian cell lines infected with budded virus. In these studies, the virus can enter cells but the viral DNA does not reach the nucleus in an expressible form.

There is also a long history of human exposure to baculoviruses. Baculoviruses are ubiquitous in nature and the indigenous load in the environment is quite high. Polyhedra counts made on cabbage taken from store shelves or collected from the field vary between 2×10^6 polyhedra/in^2 (3.1×10^{13} polyhedra/ha) on nonepizootic plots to 7×10^7 polyhedra/in^2 (1.1×10^{14} polyhedra/ha) on epizootic plots. Using these numbers, it has been estimated that a typical cole slaw serving contains an average of 1.12×10^8 polyhedra. Furthermore, baculoviruses have been used for biological insect control for more than 100 years and the use of CYD-X is not expected to significantly increase the baculovirus level in the environment. There have been no adverse effects noted as a result of either natural populations of baculoviruses or to applications of baculovirus-based pesticide products.

Finally, the mode of action associated with baculoviruses is non-toxic in nature and the formulation ingredient in CYD-X is not of toxicological concern.

Higher tier subchronic and chronic toxicity studies were not required for CYD-X Technical and CYD-X because of the known low acute toxicity potential of baculoviruses and the lack of infectivity, toxicity or pathogenicity in mammalian tissue culture studies.

3.2 Occupational/Bystander Exposure and Risk Assessment

3.2.1 Occupational

CYD-X is used as a commercial grade insecticide to control codling moth (*Cydia pomonella*) on apple trees. The product is applied using orchard air blast sprayers. Applications are repeated every 7–14 days during the growing season.

When used according to the label instructions, the potential routes of handler exposure to CpGV strain M are pulmonary, dermal and to some extent ocular. However, the PMRA does not expect that the occupational exposures from the uses of CYD-X will be of concern on the basis of the low toxicity/pathogenicity profile for CpGV strain M, and on the assumption that the precautionary labelling instructions aimed at minimizing worker exposure are adhered to by users.

Since unbroken skin is a natural barrier to microbial invasion of the human body, dermal absorption could occur only if the skin were cut, if the microbe were a pathogen equipped with mechanisms for entry through or infection of the skin, or if metabolites were produced that could be dermally absorbed. This MPCA has not been identified as a wound pathogen and there is no indication that it could penetrate intact skin of healthy individuals. Although no dermal toxicity and little dermal irritation are expected based on toxicological studies submitted in support of the MPCA and toxicological characteristics of the formulation ingredients present in the end-use formulation, all MPCAs are considered potential sensitizers. The PMRA assumes that all microorganisms contain substances that can elicit positive hypersensitivity reactions.

Although the potential for toxicity is low in individuals exposed to CpGV strain M, the presence of insect debris in the end-use product may cause irritation if inhaled or exposed to the skin or eyes. Sensitization may also occur upon repeated exposure to high concentrations of the product.

Label statements warning users that the product is a dermal and eye irritant as well as a potential sensitizer are required. To minimize exposure to mists generated while handling or applying the product, applicators, mixer/loaders, and handlers will be required to wear personal protective equipment, including a long-sleeved shirt, long pants, shoes plus socks, water-proof gloves, eye goggles and a dust/mist filtering respirator (MSH/NIOSH approval number prefix TC-21C) or a NIOSH approved respirator with any N-95, R-95, P-95 or HE filter for biological products.

3.2.2 Bystander

Overall the PMRA does not expect that bystander exposure will pose an undue risk on the basis of the low toxicity/pathogenicity profile for CpGV strain M and its associated end-use formulation and the assumption that precautionary label statements will be followed in the use of CYD-X to minimize off-target spray drift.

The label does not allow applications to turf, residential or recreational areas; therefore, non-occupational dermal exposure and risks to adults, infants, and children are low. Because the use sites are agricultural, exposure to infants and children in school, residential and daycare facilities is likely to be minimal to non-existent. Consequently, the health risk to infants and children is expected to be negligible.

3.3 Dietary Exposure and Risk Assessment

3.3.1 Food

CYD-X is used as a commercial grade insecticide to control codling moth (*C. pomonella*) on apple trees. The product is applied using orchard air blast sprayers. As codling moths are internal fruit feeders, applications are made post fruit emergence. Applications are repeated every 7−14 days during the growing season.

The application of CYD-X to apple trees after emergence of fruit is expected to result in residues of CpGV strain M on treated produce that are higher than levels naturally occurring on fruit. However, no adverse effects from dietary exposure have been attributed to natural populations of CpGV and no adverse effects were noted in oral toxicity or tissue culture studies with various baculoviruses. Furthermore, the host range associated with baculoviruses is highly specific to arthropods and there has been a long history of human exposure to baculoviruses without any observed adverse effects.

Consequently, higher tier subchronic and chronic dietary exposure studies were not required by the PMRA.

As there are no concerns for chronic risks posed by dietary exposure of the general population and sensitive subpopulations, such as infants and children, the PMRA does not require crop residue data on treated apples for CpGV strain M.

3.3.2 Drinking Water

As the Granulovirus OBs are not soluble in water and are well-retained by soil, the entry of CpGV strain M into aquatic systems via run-off and drainage is improbable. Exposure to aquatic systems would only occur via spray drift as a result of application using orchard airblast sprayers. No risks are expected from exposure to this microorganism via drinking water because exposure will be minimal and because no adverse effects resulting from exposure are expected. The CYD-X label instructs users to avoid contamination of irrigation and drinking water supplies as well as aquatic habitats through equipment cleaning or waste disposal. Users are also required to avoid runoff and spray drift. Furthermore, municipal treatment of drinking water is expected to remove the transfer of residues to drinking water. Therefore, potential exposure to CpGV strain M in surface and drinking water is minimal.

3.3.3 Acute and Chronic Dietary Risks for Sensitive Subpopulations

Calculations of acute reference doses and acceptable daily intakes are not usually possible for predicting acute and long term effects of microbial agents in the general population or to potentially sensitive subpopulations, particularly infants and children. The single (maximum hazard) dose approach to testing MPCAs is sufficient for conducting a reasonable general assessment of risk if no significant adverse effects (that is, no acute toxicity, infectivity or pathogenicity endpoints of concern) are noted in acute toxicity and infectivity tests. Based on all the available information and hazard data, the PMRA concludes that the MPCA is of low toxicity, is not pathogenic or infective to mammals, and that infants and children are likely to be no more sensitive to the MPCA than the general population. Thus, there are no threshold effects of concern and, as a result, no need to require definitive (multiple dose) testing or apply uncertainty factors to account for intra- and interspecies variability, safety factors or margins of exposure. Further factoring of consumption patterns among infants and children, special susceptibility in these subpopulations to the effects of the MPCA, including neurological effects from pre- or post-natal exposures, and cumulative effects on infants and children of the MPCA and other registered micro-organisms that have a common mechanism of toxicity, does not apply to this MPCA. As a result, the PMRA has not used a margin of exposure (safety) approach to assess the risks of this MPCA to human health.

3.4 Maximum Residue Limits

As part of the assessment process prior to the registration of a pesticide, Health Canada must determine whether the consumption of the maximum amount of residues, that are expected to remain on food products when a pesticide is used according to label directions, will not be a concern to human health. This maximum amount of residues expected is then legally established as a maximum residue limit (MRL) under the *Pest Control Products Act* for the purposes of the adulteration provision of the *Food and Drugs Act*. Health Canada sets science-based MRLs to ensure the food Canadians eat is safe.

The application of CYD-X to apple trees post fruit emergence is expected to result in residues of CpGV strain M on treated produce that are higher than levels naturally occurring on fruit. However, no adverse effects from dietary exposure have been attributed to natural populations of

CpGV and no adverse effects were noted in oral toxicity or tissue culture studies with various baculoviruses. Furthermore, the host range associated with baculoviruses is highly specific to arthropods and there has been a history of exposure to baculoviruses without any observed adverse effects. Therefore, the establishment of an MRL is not required for CpGV strain M.

3.5 Aggregate Exposure

Based on the toxicity and infectivity test data submitted and other available information, there is reasonable certainty no harm will result from aggregate exposure of residues of CpGV strain M to the general Canadian population, including infants and children, when the microbial pest control product, CYD-X, is used as directed on the label. This includes all anticipated dietary (food and drinking water) exposures and all other non-occupational exposures (dermal and inhalation) for which there is reliable information. Additionally, there have been no adverse effects from exposure to natural populations of baculoviruses in the environment.

Even if there is an increase in exposure to this microorganism from the uses of CYD-X, there should not be any increase in potential human health risk.

3.6 Cumulative Effects

The PMRA has considered available information on the cumulative effects of such residues and other substances that have a common mechanism of action. These considerations included the cumulative effects on infants and children.

Besides naturally occurring isolates of CpGV in the environment, and *Cydia pomonella* Granulovirus strain CmGV4 found in the commercial biopesticide Virosoft CP4, the PMRA is not aware of any other microorganisms, or other substances that share a common mechanism of action with this active ingredient. No cumulative effects are anticipated if the residues of CpGV strain M interact with other related isolates of this microorganism in the environment.

3.7 Incident Reports

Since 26 April 2007, registrants have been required by law to report incidents, including adverse effects to health and the environment, to the PMRA within a set time frame. Information on the reporting of incidents can be found on the Pesticide and Pest Management portion of Health Canada's website. Incidents from Canada were searched and reviewed for CpGV. As of 10 May 2011, there have been no incidents related to adverse health effects reported for products containing CpGV.

4.0 Impact on the Environment

4.1 Fate and Behaviour in the Environment

Environmental fate testing is intended to demonstrate whether an MPCA is capable of surviving or replicating in the environment to which it is applied, and could provide an indication of which non-target organisms may be exposed to the MPCA as well as provide an indication of the extent

of exposure. Environmental fate data (Tier II/III) are not normally required at Tier I, and are only triggered if significant toxicological effects in non-target organisms are noted in Tier I testing. As waiver rationales submitted to address environmental toxicology were adequate, no fate data are required to complete the environmental risk assessment of CYD-X Technical when formulated as the end-use product CYD-X.

Nevertheless, information in the published literature and other international reviews have concluded that persistence of CpGV on foliage and fruit following application is limited due to degradation by UV light. The activity of CpGV applied to apples and exposed to sunlight was measured in one study. Larval mortality was approximately 80% immediately after application but decreased to almost 0% after 300 hours of sunlight. The calculated half-life of CpGV was found to be approximately 52.2 sunlight hours. Therefore, persistence of CpGV strain M in the environment and exposure to non-target organisms are limited. Virus particles in soil, however, may persist for longer periods.

Granulovirus OBs are not soluble in water and are retained by soil. Therefore, entry of CpGV into aquatic systems via run-off or drainage is improbable. Exposure to aquatic systems and aquatic non-target organisms is only expected to occur via spray drift during product application.

4.2 Effects on Non-Target Species

4.2.1 Effects on Terrestrial Organisms

Rationales to waive environmental toxicity testing on avian species, wild mammals, terrestrial arthropods, non-arthropod invertebrates and terrestrial plants were deemed acceptable. The waiver rationales were based on reports in the published literature and in literature reviews demonstrating a lack of adverse effects on non-target organisms, the limited host range associated with CpGV and a long history of exposure without reports of adverse effects.

Because of the close relationship within the family Baculoviridae, results from studies with nucleopolyhedroviruses (NPVs) and other Granuloviruses, including other isolates of CpGV, were considered by PMRA in lieu of actual testing on non-target organisms with CpGV strain M.

In one study on honeybees, CpGV was applied by contact and oral administration in a 50% sucrose solution. No harmful effects were noted in the three days of observation after exposure. In a second study, a hive of bees was fed 1×10^{10} OBs of CpGV in 200 mL of a 50% sucrose solution. Controls were fed sucrose solution without virus. No differences were observed between treated and untreated hives in the four months following exposure.

The study results and weight of evidence in the published literature on host specificity of baculoviruses suggest that CpGV strain M has a narrow host range limited to codling moth. Given that infectivity of Granuloviruses is limited to insect species within the same family as the host in which the Granulovirus was originally isolated (in the case of CpGV strain M, infectivity is limited to the family Tortricidae), CpGV strain M would likely have a minimal impact on non-target terrestrial arthropod species.

A literature review was cited in which it was reported that oral administration of 50 mg/kg bw of *Pieris rapae* Granulovirus (PrGV) to chickens and other birds yielded no adverse effects during the 2–3 months following administration. Other reports include high single-dose levels of technical virus preparations of the *Orgyia pseudotsugata* NPV (OpNPV) fed to mallard ducks, ring-necked pheasants, and English sparrows did not product any symptoms or signs of systemic toxicity or pathogenicity, except for minor temporary weakness in a few test subjects.

Aside from laboratory tests, environmental impact studies on birds following field application of NPVs and studies on the natural occurrence of NPVs in wild birds have been conducted. After aerial application of the gypsy moth NPV, data from 23 caged quails and 53 free birds showed no differences between NPV-treated and control birds as judged by organ weights, necropsy or histopathological rankings of the condition of organs and tissues. An aerial application of the NPV from the red-headed pine sawfly, *N. lecontei*, did not cause any adverse immediate or short-term impact upon bird populations in the treated areas. Similarly, no deleterious effects on small forest songbirds were attributed to an aerial application of the spruce budworm NPV.

No studies were submitted on the effects of CpGV strain M on wild mammals and non-arthropod invertebrates. Results of published studies on other baculoviruses suggest that CpGV strain M is unlikely to infect any vertebrate cells or to produce any adverse effects in wild mammals or non-arthropod invertebrates.

In lieu of testing on terrestrial plants, a waiver rationale based on literature reviews of baculoviruses was submitted. Baculoviruses are not related to any known plant viruses and there have been no reports in the literature of baculoviruses being phytotoxic or phytopathogenic. Therefore, it is not expected that CpGV strain M will pose a risk to terrestrial plants.

More generally, baculoviruses are highly host specific. No member of the Baculoviridae family is known to infect vertebrates or plants. Granuloviruses have only been reported from members of the order Lepidoptera. Infectivity of Granuloviruses is limited to insect species within the same family as the host in which the Granulovirus was originally isolated. Cross-infectivity to alternate host species of other insect families has not been reported for Granuloviruses and, therefore, effects on even more distantly-related non-target organisms not expected to occur.

Furthermore, baculoviruses have been used for biological insect control for more than 100 years. No signs of adverse effects have been reported in avian species, wild mammals, terrestrial arthropod populations, non-arthropod invertebrates, or terrestrial plants due to either natural populations of baculoviruses or to applications of baculovirus-based pesticide products.

Based on this information, the PMRA is reasonably assured that the use of CYD-X will not present an unacceptable risk to terrestrial species including avian animals, wild mammals, arthropods (with the possible exception of members of the Tortricidae family), non-arthropod invertebrates, and terrestrial plants.

4.2.2 Effects on Aquatic Organisms

Rationales to waive environmental toxicity testing on freshwater fish, aquatic arthropods, aquatic non-arthropod invertebrates and aquatic plants were deemed acceptable. The waiver rationales were based on reports in the published literature and in literature reviews demonstrating a lack of adverse effects on non-target organisms, the limited host range associated with CpGV, limited exposure to aquatic environments and a long history of exposure without reports of adverse effects.

Because of the close relationships within the family Baculoviridae, results from studies with NPVs and other Granuloviruses on aquatic non-target organisms are considered in the assessment of CpGV strain M.

A review article was cited in which it was reported that rainbow trout and carp were immunized with *Laspeyresia pomonella* Granulovirus (CmGV; LpGV) by intramuscular injection. The fish were challenged 14 days after the first injection. Virus-specific antibodies could be detected thus demonstrating that trout and carp produce detectable antibodies to granulin. Rainbow trout were then administered LpGV at a dose of 1×10^{12} OBs/fish by force-feeding. Feces were collected daily for six days thereafter and purified by sucrose gradient centrifugation. Granulovirus was detectable in the feces for only up to three days postfeeding. Sera were also collected. No virus-specific antibodies were detected in the sera of test animals during 80 days postfeeding. The researchers concluded that no virus replication had taken place in the test fish.

A second study was cited in which 50 mg/kg bw of PrGV were applied to fish. No adverse effects were noted during the two to three month observation period. No other details were available.

Based on the cited studies, the limited host range associated with baculoviruses (see Section 4.2.1), and the limited exposure to aquatic habitats, CpGV strain M would present a low environmental risk to fish when used according to the label directions.

No studies were submitted on the effects of CpGV strain M or other baculoviruses on aquatic arthropods. However, given that infectivity of Granuloviruses is limited to insect species within the same family as the host in which the Granulovirus was originally isolated (in the case of CpGV strain M, infectivity is limited to the family Tortricidae) and that exposure to aquatic habitats is expected to be minimal, CpGV strain M would likely have a minimal impact on non-target aquatic arthropod species.

No information was submitted to address potential effects of CpGV strain M on non-arthropod invertebrates. Reports in the public literature exist, however, in which oysters were exposed to *Heliothis zea* NPV without any observations of adverse effects. In addition, the limited host range associated with baculoviruses and the limited environmental exposure to aquatic habitats indicate that the use of CpGV strain M according to label directions would not result in adverse effects in non-arthropod invertebrates.

No information on the effect of CpGV strain M on aquatic plants was submitted. Baculoviruses are not related to any known plant viruses and there have been no reports in the literature of baculoviruses being phytotoxic or phytopathogenic. Therefore, it is not expected that CpGV strain M will pose a risk to aquatic plants.

Furthermore, baculoviruses are ubiquitous in the environment and have been used for biological insect control for over 100 years without any adverse effects observed among aquatic non-target organisms. Based on these considerations, the use of CYD-X is not expected to present an unacceptable risk to aquatic species including freshwater fish, aquatic arthropods, aquatic non-arthropod invertebrates, and aquatic plants.

4.3 Incident Reports

Since 26 April 2007, registrants have been required by law to report incidents, including adverse effects to health and the environment, to the PMRA within a set time frame. Information on the reporting of incidents can be found on the Pesticide and Pest Management portion of Health Canada's website. Incidents from Canada were searched and reviewed for CpGV. As of 10 May 2011, there have been no incidents related to adverse environmental effects reported for products containing CpGV.

5.0 Value

5.1 Effectiveness Against Pests

Efficacy data were submitted and 17 trials were reviewed, all conducted on apples except for one trial on pear. CYD-X was applied at the proposed rate, as well as various higher and lower rates. In some trials, the product was applied with an adjuvant. In some trials, the product was applied in combination with one or more additional insecticides. Two to 14 applications were made at intervals of 5–14 days. Efficacy was rated in a number of ways involving first- and second-generation larval and adult populations and damaged fruit. The experimental trials demonstrated that with multiple applications, CYD-X controls codling moth larvae and causes a reduction in fruit damage due to larval feeding.

5.1.1 Acceptable Efficacy Claims

The submitted efficacy data and available information support the use of CYD-X for control of codling moth on apple at a rate of 250 mL per hectare applied in sufficient water for thorough coverage of the tree canopy.

5.2 Phytotoxicity to Host Plants

In the trials that examined host plant toxicity, no adverse effects on the host plants were observed.

5.3 Economics

No economic analysis was conducted for this product evaluation.

5.4 Sustainability

5.4.1 Survey of Alternatives

The alternatives registered in Canada for control of codling moth on apples are products that contain older chemistries such as carbaryl, methomyl, azinphos-methyl, diazinon, malathion, phosalone, phosmet and endosulfan. Pyrethroid active ingredients such as cypermethrin, deltamethrin, lambda-cyhalothrin and permethrin are registered for this use as well as newer chemistries such as acetamiprid, clothianidin, thiacloprid, spinetoram, spinosad, novaluron, methoxyfenozide, tebufenozide, *Cydia pomonella* granulosis virus (strain CMGv4), kaolin and pheromone for codling moth mating disruption.

The only products registered for use by organic growers are the pheromone, spinosad, *Cydia pomonella* granulosis virus (strain CMGv4) and kaolin.

5.4.2 Compatibility with Current Management Practices Including Integrated Pest Management

CYD-X is compatible with current management practices and works well in conjunction with area-wide mating disruption as well as other IPM strategies. Due to the species-specific nature of this product, CYD-X is not expected to have adverse effects on non-target organisms and may reduce the need for application of conventional insecticides in an IPM program.

5.4.3 Information on the Occurrence or Possible Occurrence of the Development of Resistance

Development of resistance to CpGV has been documented in other countries but to date, has not been reported in North American orchards. The virus strain in CYD-X is a Mexican isolate (strain M) which is different than the strain for the currently registered Canadian isolate of CpGV (strain CMGv4; Registration Number 26533). By alternating strains and utilizing resistance management strategies, the possible development of resistance should be reduced.

5.4.4 Contribution to Risk Reduction and Sustainability

Due to the species-specific nature of this product, CYD-X is not expected to have adverse effects on non-target organisms and may reduce the need for application of conventional insecticides in an IPM program. This product may be especially useful for organic growers.

6.0 Pest Control Product Policy Considerations

6.1 Toxic Substances Management Policy Considerations

The TSMP is a federal government policy developed to provide direction on the management of substances of concern that are released into the environment. The TSMP calls for the virtual elimination of Track 1 substances [those that meet all four criteria outlined in the policy, i.e. persistent (in air, soil, water and/or sediment), bio-accumulative, primarily a result of human activity, and toxic as defined by the *Canadian Environmental Protection Act*].

During the review process, CpGV strain M and its associated products, CYD-X Technical and CYD-X, were assessed in accordance with the PMRA Regulatory Directive DIR99-03[4] and evaluated against the Track 1 criteria. The PMRA has reached the following conclusions:

- CpGV strain M does not meet the Track 1 criteria because it is a biological organism and hence is not subject to the criteria used to define persistence, bioaccumulation and toxicity properties of chemical control products.

- There are no formulants, contaminants or impurities present in CYD-X Technical or CYD-X that meet the TSMP Track 1 criteria.

Therefore, the use of CYD-X Technical and CYD-X are not expected to result in the entry of Track 1 substances into the environment.

6.2 Formulants and Contaminants of Health or Environmental Concern

During the review process, contaminants in the technical and formulants and contaminants in the end-use products are compared against the *List of Pest control Product Formulants and Contaminants of Health or Environmental Concern* maintained in the *Canada Gazette*.[5] The list is used as described in the PMRA Notice of Intent NOI2005-01[6] and is based on existing policies and regulations including DIR99-03 and DIR2006-02,[7] and taking into consideration the Ozone-depleting Substance Regulations, 1998, of the *Canadian Environmental Protection Act* (substances designated under the Montreal Protocol).

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4 DIR99-03, *The Pest Management Regulatory Agency's Strategy for Implementing the Toxic Substances Management Policy*

5 *Canada Gazette*, Part II, Volume 139, Number 24, SI/2005-114 (2005-11-30) pages 2641–2643: *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern* and in the order amending this list in the *Canada Gazette,* Part II, Volume 142, Number 13, SI/2008-67 (2008-06-25) pages 1611-1613. *Part 1 Formulants of Health or Environmental Concern, Part 2 Formulants of Health or Environmental Concern that are Allergens Known to Cause Anaphylactic-Type Reactions and Part 3 Contaminants of Health or Environmental Con/cern.*

6 NOI2005-01, *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern under the New Pest Control Products Act*.

7 DIR2006-02, *Formulants Policy and Implementation Guidance Document*.

The PMRA has reached the following conclusions:

- No formulants or contaminants of health or environmental concern identified in the *Canada Gazette* are present in CYD-X Technical or CYD-X.

The use of formulants in registered pest control products is assessed on an ongoing basis through PMRA formulant initiatives and Regulatory Directive DIR2006-02.

7.0 Summary

7.1 Methods for Analysis of the Micro-organism as Manufactured

The product characterization data for CYD-X Technical and CYD-X are adequate to assess their potential human health and environmental risks. The technical grade active ingredient was characterized and the specifications were supported by the analyses of a sufficient number of batches to permit a registration decision.

Although a method (REN analysis) was provided for distinguishing the MPCA from other closely related *Cydia pomonella* Granuloviruses, a side-by-side comparison was not conducted to validate the method. The registrant has committed to providing this side-by-side comparative analysis of DNA REN digests.

Additional batch analysis data for microbial contaminant screens are required on four batches of CYD-X. Protocols are also required for screening and analysis of additional indicator microorganisms as identified by PMRA for baculovirus-based pesticides.

To confirm the storage stability results that have already been submitted, storage stability data on an additional three batches of CYD-X are required.

7.2 Human Health and Safety

The acute toxicity and infectivity information submitted in support of CpGV strain M were determined to be sufficiently complete to permit a decision on registration. Based on studies with other baculoviruses, CpGV strain M is expected to be of low toxicity via the oral, pulmonary, injection and dermal routes of exposure. There were no signs of infectivity or pathogenicity in mammalian tissue culture studies conducted with CpGV strain M and other baculoviruses. Although baculoviruses have not been found to be dermal or ocular irritants or sensitizers, the presence of insect debris in the end-use product may cause irritation upon inhalation or dermal or ocular exposure. Repeated exposure to the insect debris may also result in sensitization.

When handled according to the label instructions, the potential routes of handler exposure to CpGV strain M are pulmonary, dermal and to some extent ocular. However, the PMRA does not expect that the occupational exposures from its use will be of concern on the basis of the low toxicity/pathogenicity profile for CpGV strain M and associated end-use formulation. Precautionary statements on product labels and the wearing of personal protective equipment (PPE) including protective eye wear will adequately mitigate any risks from exposure.

While CYD-X has the potential to be a sensitizer, inhalation and dermal exposure is not a concern if the required dust/mist filtering respirator/mask and appropriate PPE stipulated on the end-use product label is worn by persons involved in handling CYD-X. Furthermore, precautionary labelling will alert users of the potential sensitization hazard of the end-use product.

The application of CYD-X to apple trees post fruit emergence is expected to result in residues of CpGV strain M on treated produce that are higher than levels naturally occurring on fruit. However, no adverse effects from dietary exposure have been attributed to natural populations of CpGV and no adverse effects were noted in oral toxicity or tissue culture studies with various baculoviruses. Furthermore, the host range associated with baculoviruses is highly specific to arthropods and there has been a long history of exposure to baculoviruses without any observed adverse. Therefore, the establishment of an MRL is not required for CpGV strain M.

7.3 Environmental Risk

The request to waive toxicity testing on terrestrial and aquatic non-target organisms (including avian species, wild mammals, freshwater fish, arthropods, non-arthropod invertebrates and plants) was accepted based on reports in the open scientific literature, the limited host range associated with baculoviruses in general and CpGV specifically, limited environmental persistence and exposure, and a long history of exposure to baculoviruses without reports of adverse effects.

As a general precaution, statements are required on the label to instruct users to reduce runoff, to not apply the product to aquatic systems and to prohibit handlers from contaminating irrigation or drinking water supplies and aquatic habitats. Users will be directed to not apply the product by air and application instructions to minimize spray drift will be included. Standard disposal statements will also apply.

7.3 Value

CYD-X has value in controlling codling moth, which is a major pest of apples; it can be used by organic growers and in IPM programs.

7.4 Unsupported Uses

All proposed uses were supported.

8.0 Regulatory Decision

Health Canada's PMRA, under the authority of the *Pest Control Products Act* and Regulations, has granted conditional registration for the sale and use of CYD-X Technical and CYD-X, containing the biological active ingredient CpGV strain M, for control of codling moth in apples.

An evaluation of available scientific information found that, under the approved conditions of use, the product has value and does not present an unacceptable risk to human health or the environment.

Although the risks and value have been found acceptable when all risk-reduction measures are followed, as a condition of these registrations, additional scientific information is being requested from the applicant to ensure that microbial contaminants do not exceed the established acceptable levels. For more details, refer to the Section 12 Notice associated with these conditional registrations. The applicant must submit the following information by 1 September 2013:

Product Characterization and Analysis

- Additional microbial contaminant analysis data on a minimum of four batches of CYD-X.
- Protocols for microbial contaminant analysis testing of the indicator micro-organisms.
- Stability data under storage conditions consistent with label directions on a minimum of three additional batches of CYD-X.

NOTE: The PMRA will publish a consultation document at the time when there is a proposed decision on applications to convert these conditional registrations to full registrations or on applications to renew the conditional registrations, whichever occurs first.

List of Abbreviations

bw	body weight
CmGV	codling moth Granulovirus
CpGV	*Cydia pomonella* Granulovirus
DNA	deoxyribonucleic acid
g	gram(s)
ha	hectare(s)
HE	high efficiency
HzNPV	*Helicoverpa zea* nucleopolyhedrovirus
IPM	integrated pest management
kg	kilogram(s)
L	litre(s)
LD_{90}	lethal dose 90
LpGV	*Laspeyresia pomonella* Granulovirus
MbNPV	*Mamestra brassicae* nucleopolyhedrovirus
mg	milligram(s)
mL	millilitre(s)
MPCA	microbial pest control agent
MRL	maximum residue limit
MSB	master seed bank
n/a	not applicable
NPV	nucleopolyhedrovirus
OB	occlusion body
OgNPV	*Orygia pseudotsugata* nucleopolyhedrovirus
PIB	polyhedral inclusion body
PMRA	Pest Management Regulatory Agency
PPE	Personal Protective Equipment
PrGV	*Pieris rapae* Granulovirus
REN	restriction endonuclease
TSMP	Toxic Substances Management Policy
UV	ultraviolet
v/v	volume per volume dilution
WSB	working seed bank

Appendix I Tables and Figures

Table 1 Toxicity and Infectivity of *Cydia pomonella* Granulovirus strain M and Its Associated End-use Product (CYD-X)

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference
Acute Oral Toxicity	A waiver request was submitted citing published literature on the effects of various baculoviruses on mice, guinea pigs and hamsters after oral exposure. No adverse effects were noted. The limited host range and history of exposure to baculoviruses in general were also used to support the waiver request. **WAIVER ACCEPTED**			1778684 2055229 2055489 2055396
Acute Pulmonary Toxicity and Infectivity	A waiver request was submitted citing published literature in which guinea pigs were exposed to various baculoviruses via the inhalation route. No adverse effects were noted. The limited host range, history of exposure and other studies in the published literature on baculoviruses were also used to support the waiver request. The presence of insect debris in the end-use product, however, may cause irritation if inhaled. **WAIVER ACCEPTED**			1778685 2025862 2055229 2055489 2055276 2055396
Intravenous Injection Infectivity	A waiver request was submitted citing published literature in which mice and guinea pigs were exposed to various NPVs and GVs by intraperitoneal, intravenous or subcutaneous injection. Although mortalities were observed in the test groups, the mortality rate was comparable or less frequent than in the control groups. There was no evidence of toxicity or pathogenicity. The limited host range, history of exposure and other studies in the published literature on baculoviruses were also used to support the waiver request. **WAIVER ACCEPTED**			1778687 2025862 2055223 2055229 2055489 2055396
Acute Dermal Toxicity/Irritation	A waiver request was submitted citing published literature in which guinea pigs were exposed to various baculoviruses via the dermal route. No dermal reactions were observed. The history of exposure to baculoviruses in general, non-toxic mode of action and nature of the formulation ingredient in CYD-X were also used to support the waiver request. The presence of insect debris in the technical and end-use product, however, may cause dermal irritation. **WAIVER ACCEPTED**			1778688 2055229
Eye Irritation	A waiver request was submitted citing published literature in which various baculovirus preparations were administered to the eyes of guinea pigs. No ocular reactions were observed. The history of exposure to baculoviruses in general and nature of the formulation ingredient in CYD-X were also used to support the waiver request. The presence of insect debris in the technical and end-use product, however, may cause eye irritation. **WAIVER ACCEPTED**			1778693 2055229
Dermal Sensitization	A waiver request was submitted citing the absence of hypersensitivity among employees working with the virus and within the published literature. The history of exposure to baculoviruses in general and other studies in the published literature on baculoviruses were also used to support the waiver request. The presence of insect debris in the technical and end-use product, however, may cause dermal sensitization upon repeated exposure. **WAIVER ACCEPTED**			1778690 2055229 2055188

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference
Tissue Culture	-WI-38 and Detroit 551 mammalian cells lines -exposed to CpGV strain M in the form of liberated granulin-derived virus particles at a multiplicity of infection of 58–62 LD90s	-no signs of virus replication or cytopathic effects	-bioassay of fractionated cells indicated no increase in the quantity of virus above the residual inoculum level **NOT INFECTIVE**	1778691
Tissue Culture	In addition to the submitted study, a number of published scientific studies assessing the ability of baculoviruses to enter, infect or persist in mammalian cell lines were reviewed. There were no signs of cytopathic effects.			2055375 2055380 2055396 2055366 2055277

Table 2 Toxicity to Non-Target Species

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
Terrestrial Organisms				
Vertebrates				
Birds	Oral	A waiver request was submitted citing published scientific literature on the effects of various baculoviruses on avian species. The limited host range of baculoviruses, limited environmental persistence after application of CpGV and history of exposure to baculoviruses were also used to support the waiver request. **WAIVER ACCEPTED**		1778694 2055489 2055229
Wild Mammals	A waiver request was not submitted. A rationale based on limited host range of baculoviruses, limited environmental persistence after application of CpGV and history of exposure to baculoviruses were used to waive testing on wild mammals. **NO FURTHER DATA REQUIRED**			2055489 2055229
Invertebrates				
Arthropods				
Non-target Insect Honeybees	A waiver request was submitted citing published scientific literature on the effects of CpGV on honeybees. The limited host range of baculoviruses and minimal increased environmental exposure to CpGV were also used to support the waiver request. Only a few members of the family Tortricidae are expected to be affected. **WAIVER ACCEPTED**			1778697 2055489 2055229
Non-arthropods				
Invertebrates	A waiver request was not submitted. Reports in the published scientific literature on the effects of various baculoviruses on non-arthropod invertebrates, limited host range of baculoviruses and minimal increased environmental exposure to CpGV were used to waive testing on non-arthropod invertebrates. **NO FURTHER DATA REQUIRED**			2055489 2055229

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
Plants				
Plants	A waiver request was submitted based on the fact that baculoviruses are not related to any known plant viruses and the lack of reports of literature of phytotoxicity or phytopathogenicity. The limited environmental persistence after application of CpGV and history of exposure to baculoviruses were also used to support the waiver request. **WAIVER ACCEPTED**			1778699 2055489 2055229
Aquatic Organisms				
Vertebrates				
Freshwater fish	A waiver request was submitted citing published scientific literature on the effects of various baculoviruses on freshwater fish. The limited host range of baculoviruses, limited environmental exposure to CpGV and history of exposure to baculoviruses were also used to support the waiver request. **WAIVER ACCEPTED**			1778695 2055489 2055229
Estuarine and marine fish	A waiver request was not submitted. The limited host range of baculoviruses and limited environmental exposure to CpGV were used to waive testing on estuarine and marine fish. **NO FURTHER DATA REQUIRED**			2055489 2055229
Invertebrates				
Arthropods	A waiver request was not submitted. The limited host range of baculoviruses and limited environmental exposure to CpGV were used to waive testing on aquatic arthropods. **NO FURTHER DATA REQUIRED**			2055489 2055229
Non-Arthropod Invertebrates	A waiver request was not submitted. Reports in the published scientific literature on the effects of various baculoviruses on non-arthropod invertebrates, limited host range of baculoviruses and limited environmental exposure to CpGV were used to waive testing on non-arthropod invertebrates. **NO FURTHER DATA REQUIRED**			2055489 2055229
	Plants			
Plants	A waiver request was not submitted. Aquatic plant testing was waived based on the fact that baculoviruses are not known to be related to any plant viruses, the lack of reports of phytoxocitiy or phytopathogenicity, and limited environmental exposure to CpGV. **NO FURTHER DATA REQUIRED**			2055489 2055229

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A. List of Studies/Information Submitted by Registrant

1.0 Characterization and Analysis

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1778652 2009, CYD-X chemistry package, DACO: M2.10.1, M2.10.2, M2.12, M2.7.1, M2.8, M2.9.2, M2.9.3 CBI

1778653 2007, Comparative restriction analysis of CpGV (Virosoft) with CpGV (Neustadt Mexican isolate), DACO: M2.7.1 CBI

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1778656 2009, Biological properties, DACO: M2.7.2

1778680 2009, Product characterization, DACO: M2.1, M2.2, M2.3, M2.4, M2.5, M2.6

1778681 2009, CYD-X product chemistry, DACO: M2.10.1, M2.10.2, M2.12, M2.8, M2.9.1, M2.9.2, M2.9.3 CBI

1778682 1991, Waiver request - stability, DACO: M2.11

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2.0 Human and Animal Health

1778683 2009, Infectivity and toxicity summary, DACO: M4.2.1

1778684 1991, Waiver request for acute oral infectivity and toxicity, DACO: M4.2.2

1778685 1991, Waiver request for acute pulmonary infectivity and toxicity, DACO: M4.2.3

1778686 2009, Acute infectivity (IV or IP) summary, DACO: M4.3.1

1778687 1991, Waiver request - additional toxicity, DACO: M4.3.2

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1778689 2009, Irritation summary, DACO: M4.5.1

1778690 1991, Waiver request for reporting of hypersensitivity incidence, DACO: M4.6

1778691 1994, Human cell culture assay for codling moth granulosis virus replication, DACO: M4.7

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3.0 Environment

1778694 1991, Waiver request for avian oral, DACO: M9.2.1

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4.0 Value

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1778701 2007, Efficacy of CYD-X and Isomate CM/OFM TT against the codling moth, *Cydia pomonella*, in Pennsylvania apple orchards 2006, DACO: M10.2.2

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B. Additional Information Considered

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2.0 Environment

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