Proposed Registration Decision PRD2022-12

Attenuated *Cucumber green mottle mosaic virus* strain ON-BM3; Al-Bio10 and Cuc-GUARD

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Publications
Pest Management Regulatory Agency
Health Canada
2720 Riverside Drive
A.L. 6607 D
Ottawa, Ontario K1A 0K9

Internet: canada.ca/pesticides
pmra.publications-arla@hc-sc.gc.ca
Facsimile: 613-736-3758
Information Service:
1-800-267-6315 or 613-736-3799
pmra.info-arla@hc-sc.gc.ca

Canada

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Overview

Proposed registration decision for attenuated *Cucumber green mottle mosaic virus* strain ON-BM3; Al-Bio10

Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the *Pest Control Products Act*, is proposing registration for the sale and use of Attenuated CGMMV strain ON-BM3; Al-Bio10 Technical and Cuc-GUARD, containing the technical grade active ingredient attenuated *Cucumber green mottle mosaic virus* (CGMMV) strain ON-BM3; Al-Bio10, to protect cucumber plants from virulent forms of CGMMV.

An evaluation of available scientific information found that, under the approved conditions of use, the health and environmental risks and the value of the pest control products are acceptable.

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 attenuated CGMMV strain ON-BM3; Al-Bio10 and Cuc-GUARD.

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. 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 Health Canada regulates pesticides, the assessment process and risk-reduction programs, please visit the Pesticides section of Canada.ca.

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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."

Before making a final registration decision on attenuated CGMMV strain ON-BM3; Al-Bio10 and Cuc-GUARD, Health Canada's PMRA will consider any comments received from the public in response to this consultation document.[3] Health Canada will then publish a Registration Decision[4] on attenuated CGMMV strain ON-BM3; Al-Bio10 and Cuc-GUARD, which will include the decision, the reasons for it, a summary of comments received on the proposed registration decision and Health Canada's response to these comments.

For more details on the information presented in this Overview, please refer to the Science Evaluation of this consultation document.

What is attenuated *Cucumber green mottle mosaic virus* strain ON-BM3; Al-Bio10?

Attenuated CGMMV strain ON-BM3; Al-Bio10 is a modified form of CGMMV. This modified strain protects greenhouse cucumbers against aggressive forms of the virus through cross-protection, which is a plant defense mechanism against viral infection.

Health considerations

Can approved uses of attenuated CGMMV strain ON-BM3; Al-Bio10 affect human health?

Attenuated CGMMV strain ON-BM3; Al-Bio10 is unlikely to affect your health when Cuc-GUARD is used according to the label directions.

Potential exposure to attenuated CGMMV strain ON-BM3; Al-Bio10 may occur when handling and applying Cuc-GUARD. When assessing health risks, several key factors are considered:

- the microorganism's biological properties (for example, infection cycle);
- reports of any adverse incidents;
- its potential to cause disease or toxicity; and
- the level to which people may be exposed relative to exposures already encountered in nature to other isolates of this microorganism.

The levels used to assess risks are established to protect the most sensitive human population (for example, children and nursing mothers). As such, sex and gender are taken into account in the risk assessment. Only uses that are determined as having no health risks of concern are considered acceptable for registration.

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3 "Consultation statement" as required by subsection 28(2) of the *Pest Control Products Act*.

4 "Decision statement" as required by subsection 28(5) of the *Pest Control Products Act*.

Waiver rationales supported by published scientific literature were submitted in lieu of toxicity and pathogenicity testing. Attenuated CGMMV strain ON-BM3; Al-Bio10 Technical and Cuc-GUARD are considered to be of low toxicity via the oral, pulmonary, and dermal routes and the microbial pest control agent (MPCA) is not considered infective or pathogenic. The end-use product and technical grade active ingredient are considered to be irritating to the eyes and skin, and are considered potential sensitizers.

Residues in water and food

Dietary risks from food and water are acceptable.

Residues of attenuated CGMMV strain ON-BM3; Al-Bio10 on treated crops are possible at the time of harvest. However, the technical grade active ingredient and end-use product are considered to be of low toxicity and the MPCA is not considered infective or pathogenic. In addition, the likelihood of attenuated CGMMV strain ON-BM3; Al-Bio10 contaminating drinking water is expected to be low as the label has the necessary mitigation measures to limit contamination of drinking water from the proposed uses of Cuc-GUARD. Consequently, dietary risks are acceptable for all segments of the population, including infants, children, adults and seniors.

Occupational risks from handling Cuc-GUARD

Occupational risks are acceptable when Cuc-GUARD is used according to label directions, which include protective measures.

Workers handling Cuc-GUARD can come into direct contact with attenuated CGMMV strain ON-BM3; Al-Bio10 on the skin, by inhalation, or in the eyes. To protect workers from exposure to Cuc-GUARD, the label states that workers must wear personal protective equipment, including long-sleeved shirt, long pants, protective eyewear (goggles), waterproof gloves, socks and shoes and a NIOSH-approved particulate filtering facepiece respirator with any N, R or P filter. The product label includes measures to restrict access to the treated area for 4 hours or until sprays have settled. The occupational risks are acceptable when the precautionary statements on the label are observed.

Risks in residential and other non-occupational environments

Estimated risk for non-occupational exposure is acceptable.

Cuc-GUARD is proposed for commercial use as a high-pressure spray in greenhouses only. Residential and non-occupational exposure to Cuc-GUARD is therefore expected to be low when label directions are observed. Consequently, the risk to residents and the general public is acceptable.

Environmental considerations

What happens when attenuated CGMMV strain ON-BM3; Al-Bio10 is introduced into the environment?

Environmental risks are acceptable.

Cucumber green mottle mosaic virus is a plant pathogen that causes disease in cucumber and other cucurbit crops. The virus belongs to the genus *Tobamovirus* in the family Virgaviridae. Since first described in 1935 in England, CGMMV has spread worldwide to other areas of Europe, Asia, Australia, Africa, and North America. *Cucumber green mottle mosaic virus* can spread readily by contact as its virions are stable and have been shown to remain infectious on contaminated surfaces for long periods.

Minimal environmental exposure to attenuated CGMMV strain ON-BM3; Al-Bio10 is expected following the proposed use in greenhouses.

Waiver rationales supported by published scientific literature were submitted in lieu of toxicity and pathogenicity testing on birds, wild mammals, fish, terrestrial and aquatic arthropods, honeybees, non-arthropod invertebrates, microorganisms, and terrestrial and aquatic plants. While the MPCA may be infective to certain non-target plants, it is not expected to cause significant disease in these plants. The technical grade active ingredient and end-use product are considered to be of low toxicity and not pathogenic or infective to other non-target organisms. Furthermore, exposure to outdoor environments is expected to be minimal since the use is restricted to greenhouses, therefore, significant adverse effects to non-target organisms are not expected.

Based on a critical review of data and published scientific literature submitted by the applicant, the risks to birds, wild mammals, fish, terrestrial and aquatic arthropods, honeybees, non-arthropod invertebrates, microorganisms, and terrestrial and aquatic plants are acceptable when Cuc-GUARD is applied according to directions on the label.

Value considerations

What is the value of Cuc-GUARD?

Cuc-GUARD protects greenhouse cucumbers against aggressive strains of CGMMV.

Cucumber green mottle mosaic virus is a pathogen that causes a serious disease of greenhouse-grown cucumbers. *Cucumber green mottle mosaic virus* is a relatively recent and rapidly spreading pathogen in Canada that results in reduced crop yield, quality and marketability. There are no products available to control this disease and growers rely on greenhouse sanitation practices to exclude the virus and prevent infection of cucumber plants. Cuc-GUARD is compatible with current greenhouse integrated pest management practices.

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 being proposed on the labels of Attenuated CGMMV strain ON-BM3; Al-Bio10 Technical and the end-use product, Cuc-GUARD to address the potential risks identified in this assessment are as follows.

Key risk-reduction measures – Human Health

The signal words 'POTENTIAL SENSITIZER', 'CAUTION EYE IRRITANT', and 'CAUTION SKIN IRRITANT' will appear on the primary display panel of the labels. Also, workers handling or applying Cuc-GUARD must wear a long-sleeved shirt, long pants, protective eyewear (goggles), waterproof gloves, socks and shoes and a NIOSH-approved particulate filtering facepiece respirator with any N, R or P filter. Furthermore, all unprotected workers are restricted from entering treated areas during application and for 4 hours following application or until sprays have settled.

Key risk-reduction measures – Environment

The end-use product label will include environmental precaution statements to reduce the potential for contamination of aquatic systems from the use of Cuc-GUARD.

Next steps

Before making a final registration decision on attenuated CGMMV strain ON-BM3; Al-Bio10 and Cuc-GUARD, Health Canada's PMRA will consider any comments received from the public in response to this consultation document. Health Canada will accept written comments on this proposal up to 45 days from the date of publication of this document. Please forward all comments to Publications (contact information on the cover page of this document). Health Canada will then publish a Registration Decision, which will include its decision, the reasons for it, a summary of comments received on the proposed decision and Health Canada's response to these comments.

Other Information

When the Health Canada makes its registration decision, it will publish a Registration Decision on attenuated CGMMV strain ON-BM3; Al-Bio10 and Cuc-GUARD (based on the Science Evaluation of this consultation document). In addition, the test data referenced in this consultation document will be available for public inspection, upon application, in the PMRA's Reading Room. For more information, please contact the PMRA's Pest Management Information Service.

Science evaluation

Attenuated CGMMV strain ON-BM3; Al-Bio10 and Cuc-GUARD

1.0 The active ingredient, its properties and uses

1.1 Identity of the active ingredient

Active microorganism	Attenuated CGMMV strain ON-BM3; Al-Bio10
Function	Non-parasitic plant disease control – for the control of *Cucumber green mottle mosaic virus*
Binomial name	*Cucumber green mottle mosaic virus*
Taxonomic designation[1]	
Superkingdom	Viruses
Clade	Riboviria
Kingdom	Orthornavirae
Phylum	Kitrinoviricota
Class	Alsuviricetes
Order	Martellivirales
Family	Virgaviridae
Genus	*Tobamovirus*
Species	*Cucumber green mottle mosaic virus*
Strain	ON-BM3; Al-Bio10
Patent Status information	Canadian Patent Status: Patent Application: CA 3017465 English Title: Biological Control of Cucumber green mottle mosaic virus Filed Date: 2018-09-14 Open to Public Inspection: 2020-03-04 Issued: Not yet issued United States Patent Status: Publication No.: US 2020/0087676 A1 Title of the Invention: Biological Control of Cucumber green mottle mosaic virus Publication Date: 19 March 2020
Minimum purity of active	Technical grade active ingredient: minimum of 2×10^{10} particles/mL Cuc-GUARD End-use Product: minimum of 2×10^{8} particles/mL

Identity of relevant impurities of toxicological and/or environmental significance	The technical grade active ingredient 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.

[1] National Center for Biotechnology Information - Taxonomy Browser (https://www.ncbi.nlm.nih.gov/taxonomy)

1.2 Physical and chemical properties of the active ingredient and end-use product

Technical grade active ingredient – Attenuated CGMMV strain ON-BM3; Al-Bio10 Technical

Property	Result
Colour	Light to dark green
Physical State	Liquid
Solubility	Soluble in water
pH (1%)	6.9–7.1
Relative Density	1.008–1.012 g/cm^3 at 2°C

End-use product – Cuc-GUARD

Property	Result
Colour	Light to dark green
Physical State	Liquid
Solubility	Soluble in water
pH (1%)	6.9–7.1
Relative Density	1.1–1.3 g/cm^3 at 24°C

1.3 Directions for use

Cuc-GUARD is applied once per crop cycle to two to three week old cucumber plants. Cucumber plants must be free of CGMMV at the time of application. Cuc-GUARD is applied as a high pressure spray at 4 to 8 L product/ha. Crop maintenance activities soon after application can be expected to foster more rapid and even inoculation of the modified CGMMV strain ON-BM3, Al-Bio10.

1.4 Mode of action

Cuc-GUARD protects cucumber plants by means of cross-protection, which is essentially based on the similarity of nucleotide sequences and coat proteins of the modified CGMMV strain ON-BM3, Al-Bio10and aggressive forms of CGMMV. Once cucumber plants are infected with the CGMMV strain ON-BM3, Al-Bio10, it is thought that multiplication of RNA by aggressive forms of the virus is prevented.

2.0 Methods of analysis

2.1 Methods for identification of the microorganism

Acceptable methodologies for detection, isolation and enumeration of the active ingredient, attenuated CGMMV strain ON-BM3; Al-Bio10, were submitted by the applicant. Attenuated CGMMV strain ON-BM3; Al-Bio10 has been fully characterized with respect to the origin of the strain, natural occurrence of the wildtype strain and biological properties. Attenuated CGMMV strain ON-BM3; Al-Bio10 is a CGMMV strain native to Ontario, Canada, modified via site-directed mutagenesis that introduced nine point mutations resulting in nine distinct amino acid substitutions. Attenuated CGMMV strain ON-BM3; Al-Bio10 is a positive-strand RNA virus and can be identified to the strain level using a reverse transcription polymerase chain reaction (RT-PCR) method and the modifications can be detected using an amplification refractory mutation system (ARMS) RT-PCR method.

2.2 Methods for establishment of purity of seed stock

Attenuated CGMMV strain ON-BM3; Al-Bio10 has been deposited into the American Type Culture Collection and is stored under patent deposit number PTA-126903. The strain is maintained in an acceptable manner in order to maintain purity, viability, and genetic stability.

2.3 Methods to define the content of the microorganism in the manufactured material used for the production of formulated product

The guarantees of the technical grade active ingredient and the end-use product are expressed in units of particles/mL. Representative data on four batches of the technical grade active ingredient and six batches of the end-use product were submitted. The methods for determining the concentration of virus particles were adequately described.

2.4 Methods to determine and quantify residues (viable or non-viable) of the active microorganism and relevant metabolites

As noted above, acceptable methods are available to enumerate the microorganism and to distinguish this microbial pest control agent (MPCA) from other strains of CGMMV.

2.5 Methods for determination of relevant impurities in the manufactured material

The quality assurance procedures used to limit contaminating microorganisms during the manufacture of Attenuated CGMMV strain ON-BM3; Al-Bio10 Technical and Cuc-GUARD are acceptable. Manufacturing is conducted under controlled laboratory conditions and procedures include cleaning/disinfection of all equipment.

The absence of human pathogens and below-threshold levels of contaminating microorganisms were shown in the microbial screening of batches of Cuc-GUARD using standard microbiological methods for detecting and enumerating microbial contaminants of concern.

All batches of Cuc-GUARD conform to the limits set out in the Organisation for Economic Co-operation and Development (OECD) issue paper on microbial contaminants for microbial pest control products [ENV/JM/MONO(2011)43].

2.6 Methods to determine storage stability, shelf-life of the microorganism

Storage stability data were provided for Cuc-GUARD. Results support a storage period of 12 weeks at 4°C.

3.0 Impact on human and animal health

3.1 Toxicity and infectivity summary

3.1.1 Testing

Toxicity and infectivity test data requirements were addressed via acceptable scientific waiver rationales. Therefore, no human health and safety test data were submitted by the applicant.

3.1.2 Additional information

Scientific rationales were submitted to waive the requirement for acute oral infectivity and toxicity, acute pulmonary infectivity and toxicity, and intraperitoneal infectivity testing on Attenuated CGMMV strain ON-BM3; Al-Bio10 Technical. A scientific waiver rationale to waive the requirements for acute dermal toxicity and primary dermal irritation for the end-use product Cuc-GUARD was also submitted. The rationales used to waive the requirements for human health and safety testing were based on the following:

- It is currently accepted that a strict separation exists between plant and vertebrate viruses regarding their host range and pathogenicity, and that plant viruses are believed to infect only plants. Accordingly, plant viruses are not considered to present any potential pathogenic threats to humans or other vertebrates, although some plant viruses may replicate within the bodies of insect hosts that are acting as transmission vectors.
- In contrast to mammalian viruses, plant pathogenic viruses cannot propagate in humans and other vertebrate animals, generally due to the absence of specific receptors for recognition and penetration into host cells.
- The attenuated plant virus is a weakened version of the wildtype virulent strain and grows and propagates in the plant in the same manner as the infectious strain.
- CGMMV is very stable in the environment, can remain infectious for years in water or soil, and is found widely in the environment, including on vegetables and fruits. Livestock animals and humans are regularly exposed to this virus with no reports of adverse effects.
- The formulation ingredients used to prepare Attenuated CGMMV strain ON-BM3; Al-Bio10 Technical and Cuc-GUARD are considered to be of low toxicity and are either non-irritating or causes moderate irritation to the skin.

Furthermore, viruses are not capable of replication or metabolism outside of a suitable host, are not themselves toxic and the host used to produce the technical grade active ingredient does not produce toxins. Therefore, the technical grade active ingredient is considered to be of low toxicity.

A search by PMRA of the published literature in the National Center for Biotechnology Information search engine using the keywords 'Cucumber green mottle mosaic virus' found no listing of incidents, adverse or otherwise, involving the *Cucumber green mottle mosaic virus* and humans or other mammals.

The rationales were acceptable. Accordingly, Attenuated CGMMV strain ON-BM3; Al-Bio10 Technical and Cuc-GUARD are considered to be of low toxicity via the oral, pulmonary and dermal routes and not infective or pathogenic to humans or other mammals. In the absence of product specific test data, however, Attenuated CGMMV strain ON-BM3; Al-Bio10 Technical and Cuc-GUARD are considered to be skin and eye irritants.

The applicant has stated that during the discovery and preparation of Attenuated CGMMV strain ON-BM3; Al-Bio10 Technical, no adverse effects to workers were documented. Therefore, it can be concluded that no incidents of hypersensitivity have been reported by the applicant.

3.1.3 Incident reports related to human and animal health

Attenuated CGMMV strain ON-BM3; Al-Bio10 is a new active ingredient pending registration for use in Canada, and as of 15 March 2022, no incident reports had been submitted to the PMRA.

3.1.4 Hazard analysis

The data package submitted in support of registering Attenuated CGMMV strain ON-BM3; Al-Bio10 Technical and Cuc-GUARD was reviewed from the viewpoint of human health and safety and was determined to be acceptable.

Attenuated CGMMV strain ON-BM3; Al-Bio10 Technical is considered to be of low toxicity by the oral and pulmonary routes, and is not considered pathogenic or infective. Cuc-GUARD is not considered toxic by the dermal route. Because eye and dermal irritation testing were not conducted, the technical grade active ingredient and end-use product labels will include the standard PPE for eye and skin protection and the hazard statements 'CAUTION: EYE IRRITANT' and 'CAUTION: SKIN IRRITANT'. No reports of hypersensitivity incidents among workers were reported by the applicant nor was a dermal sensitization study conducted. However, all MPCAs are considered potential sensitizers and, consequently, the hazard statement "POTENTIAL SENSITIZER" will appear on the principal display panel of the technical grade active ingredient and end-use product labels. The statement "May cause sensitization. Avoid contact with skin, eyes or clothing." is also required on the secondary panel of the technical grade active ingredient and end-use product labels under the "PRECAUTIONS" section.

Higher tier subchronic and chronic toxicity studies were not required because the technical grade active ingredient and end-use product are considered to be of low toxicity and not infective or pathogenic to humans or other mammals.

Within the available scientific literature, there are no reports that suggest CGMMV has the potential to cause adverse effects on the endocrine system of animals. Based on the weight of evidence of available data, no adverse effects to the endocrine or immune systems are anticipated for this MPCA.

3.2 Occupational, residential and bystander risk assessment

3.2.1 Occupational and postapplication exposure and risk

When handled according to the label instructions, the potential for dermal, eye and inhalation exposure for handlers, mixer/loaders and applicators exists. 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 was a pathogen equipped with mechanisms for entry through or infection of the skin, or if metabolites were produced that could be dermally absorbed. *Cucumber green mottle mosaic virus*, including attenuated CGMMV strain ON-BM3; Al-Bio10, has not been identified as a dermal wound pathogen and there is no indication that it could penetrate intact skin of healthy individuals. Furthermore, Attenuated CGMMV strain OM-BM3; Al-Bio10 Technical is considered to be of low toxicity via the oral, pulmonary and dermal routes of exposure and not pathogenic or infective. In lieu of testing, the PMRA considers all microorganisms as mild ocular and dermal irritants; therefore the end-use product and technical grade active ingredient may cause eye and skin irritation. The PMRA also assumes that all microbes contain substances that can elicit positive hypersensitivity reactions.

Risk mitigation measures such as personal protective equipment (PPE) including a long-sleeved shirt, long pants, protective eyewear (goggles), waterproof gloves, socks and shoes and a NIOSH-approved particulate filtering facepiece respirator with any N, R or P filter are required to minimize exposure and protect handlers, mixers/loaders and applicators. Furthermore, all unprotected workers are prohibited from entering treated areas where Cuc-GUARD has been applied for 4 hours or until sprays have settled.

Label warnings, restrictions and risk mitigation measures are adequate to protect users of Cuc-GUARD. Overall, occupational risks to workers are acceptable when the precautionary statements on the labels are followed which include PPE.

3.2.2 Residential and bystander exposure and risk

Cuc-GUARD is proposed for use in greenhouses only. This use is not expected to result in significant residential and bystander exposure due to drift. Also, Attenuated CGMMV strain ON-BM3; Al-Bio10 Technical is considered to be of low toxicity and the MPCA, attenuated CGMMV strain ON-BM3; Al-Bio10, is not considered to be pathogenic or infective. Consequently, the health risks to bystanders and individuals in residential areas are acceptable.

3.3 Dietary exposure and risk assessment

3.3.1 Food

While the proposed use pattern may result in dietary exposure with possible residues in or on agricultural commodities, the risks from consuming food crops treated with Cuc-GUARD are acceptable because Cuc-GUARD, containing attenuated CGMMV strain ON-BM3; Al-Bio10, is considered to be of low toxicity, and not pathogenic or infective to humans or other mammals.

Consequently, there is no health risk for the general population, including infants and children, or animals.

3.3.2 Drinking water

Dietary exposure from drinking water is expected to be low as the label has the necessary mitigation measures to limit contamination of drinking water from the proposed uses of Cuc-GUARD in greenhouses. The label will instruct users not to contaminate irrigation or drinking water supplies or aquatic habitats through equipment cleaning or waste disposal and to not allow effluent or runoff from greenhouses containing this product to enter lakes, streams, ponds or other waters. Municipal treatment of drinking water is also expected to further reduce the transfer of residues to drinking water. Furthermore, attenuated CGMMV strain ON-BM3; Al-Bio10 is considered to be of low toxicity, and not pathogenic or infective to humans or other mammals. Consequently, the health risks from residues of attenuated CGMMV strain ON-BM3; Al-Bio10 in drinking water are acceptable.

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 (in other words, 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 attenuated CGMMV strain ON-BM3; Al-Bio10 is of low oral toxicity, is not pathogenic or infective to mammals, and that infants and children are likely to be no more sensitive to this MPCA than the general population. Thus, there are no threshold effects of concern and, as a result, there is 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 this MPCA, including neurological effects from pre- or post-natal exposures, and cumulative effects on infants and children of the MPCA and other registered microorganisms 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 attenuated CGMMV strain ON-BM3; Al-Bio10 to human health.

3.3.4 Aggregate exposure and risk

Aggregate exposure is the total exposure to a single pesticide that may occur from food, drinking water, residential and other non-occupational sources, and from all known or plausible exposure routes (oral, dermal and inhalation).

In an aggregate risk assessment, the combined potential risk associated with food, drinking water and various residential exposure pathways is assessed. A major consideration is the likelihood of co-occurrence of exposures. Additionally, only exposures from routes that share common toxicological endpoints can be aggregated.

Cuc-GUARD is considered to be of low toxicity by the oral, pulmonary and dermal routes and the end-use product will not be applied near or to drinking water. When the end-use product is used as labelled, there is reasonable certainty that no harm will result from aggregate exposure of residues of attenuated CGMMV strain ON-BM3; Al-Bio10.

3.3.5 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 specified 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 specifies science-based MRLs to ensure the food Canadians eat is safe.

Residues of attenuated CGMMV strain ON-BM3; Al-Bio10 on treated food crops are possible at the time of harvest. Dietary risk to humans from the proposed use of Cuc-GUARD is acceptable due to the low toxicity profile of attenuated CGMMV strain ON-BM3; Al-Bio10. In addition, the likelihood of residues contaminating drinking water supplies is negligible to non-existent. Therefore, the PMRA has determined that specification of an MRL under the Pest Control Products Act is not required for attenuated CGMMV strain ON-BM3; Al-Bio10.

3.4 Cumulative assessment

The *Pest Control Products Act* requires that the PMRA consider the cumulative exposure to pesticides with a common mechanism of toxicity. In its assessment of common mechanism of toxicity, the PMRA considers both the taxonomy of MPCAs and the production of any potentially toxic metabolites. For the current evaluation, the PMRA has determined that attenuated CGMMV strain ON-BM3; Al-Bio10 does not share a common mechanism of toxicity with other MPCAs. Therefore, there is no requirement for a cumulative health risk assessment at this time.

4.0 Impact on the environment

4.1 Fate and behaviour in the environment

No studies were submitted to address the environmental fate and behaviour of attenuated CGMMV strain ON-BM3; Al-Bio10, however, 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.

Cucumber green mottle mosaic virus is a plant pathogen that causes disease in cucumber and other cucurbit crops. The virus belongs to the genus *Tobamovirus* in the family Virgaviridae. Since first described in 1935 in England, CGMMV has spread worldwide to other areas of Europe, Asia, Australia, Africa, and North America. *Cucumber green mottle mosaic virus* can spread readily by contact as its virions are stable and have been shown to remain infectious on contaminated surfaces for long periods.

The proposed use of Cuc-GUARD is limited to greenhouse use sites. The intended applications are foliar. Although the greenhouse use precludes any direct exposure to outdoor environments, exposure to attenuated CGMMV strain ON-BM3; Al-Bio10 may occur through human activity such as composting of plant waste and water management practices. Afterwards, the dispersal of attenuated CGMMV strain ON-BM3; Al-Bio10 should be limited to the movement of treated plant materials, natural vectors (for example, insects), and to a limited extent runoff.

In the event that attenuated CGMMV strain ON-BM3; Al-Bio10 does reach outdoor environments, the organism is expected to behave as it would in nature. Evidence indicates that CGMMV is stable in the environment and infectious particles can be found in terrestrial and aquatic environments. However, the use of Cuc-GUARD in greenhouses only is not expected to increase these levels due to the limited exposure to outdoor environments and the apparent reduced virulence of attenuated CGMMV strain ON-BM3; Al-Bio10 in host plants.

Overall, the proposed greenhouse use of Cuc-GUARD is not expected to significantly increase natural populations of CGMMV in outdoor terrestrial or aquatic environments.

4.2 Effects on non-target species

PMRA has a four-tiered approach to environmental testing of microbial pesticides. Tier I studies consist of acute studies on up to seven broad taxonomic groups of non-target organisms exposed to a maximum hazard or Maximum Challenge Concentration of the MPCA. The Maximum Challenge Concentration is generally derived from the amount of the MPCA, or its toxin, expected to be available following application at the maximum recommended label rate multiplied by a safety factor. Tier II studies consist of environmental fate (persistence and dispersal) studies as well as additional acute toxicity testing of MPCAs. Tier III studies consist of chronic toxicity studies (life cycle studies), as well as definitive toxicity testing (for example, LC_{50}, LD_{50}). Tier IV studies consist of experimental field studies on toxicity and fate, and are required to determine whether adverse effects are realized under actual use conditions.

The type of environmental risk assessment conducted on MPCAs varies depending on the tier level that was triggered during testing. For many MPCAs, Tier I studies are sufficient to conduct environmental risk assessments. Tier I studies are designed to represent “worst-case” scenarios where the exposure conditions greatly exceed the expected environmental concentrations. The absence of adverse effects in Tier I studies are interpreted as minimal risk to the group of non-target organisms. However, higher tiered studies will be triggered if significant adverse effects on non-target organisms are identified in Tier I studies. These studies provide additional information that allows the PMRA to refine the environmental risk assessments. In the absence of adequate environmental fate and/or field studies, a screening level risk assessment can be performed to determine if the MPCA is likely to pose a risk to a group of non-target organisms.

The screening level risk assessment uses simple methods, conservative exposure scenarios (for example, direct application at a maximum application rate) and sensitive toxicity endpoints. A risk quotient (RQ) is calculated by dividing the exposure estimate by an appropriate toxicity value (RQ = exposure/toxicity), and the risk quotient is then compared to the level of concern (level of concern = 1 for most species, 0.4 for acute risk to pollinators, and 2 for glass plate studies using the standard beneficial arthropod test species, *Typhlodromus pyri* and *Aphidius rhopalosiphi*; level of concern = 1 is used for higher tier tests of the standard arthropod test species and for other arthropod test species).

If the screening level risk quotient is below the level of concern, the risk is considered negligible and no further risk characterization is necessary. If the screening level risk quotient is equal to or greater than the level of concern, then a refined risk assessment is performed to further characterize the risk. A refined assessment takes into consideration more realistic exposure scenarios (environmental fate and/or field testing results). Refinements may include further characterization of risk based on exposure modelling, monitoring data, results from field or mesocosm studies, and probabilistic risk assessment methods. Refinements to the risk assessment may continue until the risk is adequately characterized or no further refinements are possible.

4.2.1 Effects on terrestrial and aquatic organisms

Acceptable scientific rationales based on published scientific literature were submitted in lieu of

Tier I testing requirements on non-target birds, wild mammals, fish, terrestrial and aquatic arthropods including honeybees, non-arthropod invertebrates, terrestrial and aquatic plants and microorganisms.

The rationales were based on the following:

- The preparation of attenuated CGMMV strain ON-BM3; Al-Bio10 using site-directed mutagenesis is not expected to cause the MPCA to differ from the virulent wildtype strain with respect to its activity in the environment.
- The proposed pattern of use for Cuc-GUARD is for greenhouse cucumbers only. Therefore, it will not be applied in an environment outside a greenhouse.

- CGMMV is ubiquitous in the environment, including rivers and irrigation systems, with no reports of adverse effects in non-target organisms other than plants. Plant viruses including CGMMV are commonly consumed and passed through the alimentary tracts of wildlife in the environment without any observable impact on the health of these animals.
- Plant viruses, such as members of the genus *Tobamovirus*, are easily transmitted by chewing mouthparts of insects as well as legs and feet touching infected plants and landing on healthy plants with no apparent adverse effects on the insects.
- A study of the spread of CGMMV via pollination under protected cropping conditions observed the honey bee and its response to exposure to CGMMV in pollen. The study found that CGMMV can be transmitted by honey bees without any harmful effects to the bees.
- The majority of plant species infected by CGMMV are in the family Cucurbitaceae. CGMMV may replicate in non-target plants without causing symptoms of infection. These non-symptomatic plants, however, do contain CGMMV inoculum that may infect a susceptible host plant.
- The major risk of cross protection is that mild strains may become virulent ones by reverse mutations. One solution to reduce such risk is to create mild strains with two or more mutations. The Attenuated CGMMV strain ON-BM3; Al-Bio10 Technical has been developed with nine point mutations resulting in nine distinct amino acid substitutions.

Furthermore, viruses are not capable of replication or metabolism outside of a suitable host, are not themselves toxic and the host used to produce the technical grade active ingredient does not produce toxins. Therefore, the technical grade active ingredient is considered to be of low toxicity.

As discussed above, it is currently accepted that a strict separation exists between plant and vertebrate viruses regarding their host range and pathogenicity, and that plant viruses are believed to infect only plants. Accordingly, plant viruses are not considered to present any potential pathogenic threats to humans or other vertebrates, although some plant viruses may replicate within the bodies of insect hosts that are acting as transmission vectors.

A search by PMRA of the published literature in the National Center for Biotechnology Information search engine using the keywords ‘Cucumber green mottle mosaic virus’ found no listing of incidents, adverse or otherwise, involving the CGMMV and birds, wild mammals, terrestrial arthropods, honey bees, or microorganisms.

Based on all the available data and information on the effects of Attenuated CGMMV strain ON-BM3; Al-Bio10 Technical to terrestrial non-target organisms, there is reasonable certainty that no harm will be caused to birds, wild mammals, fish, terrestrial and aquatic arthropods including honey bees, non-arthropod invertebrates, terrestrial and aquatic plants and microorganisms from the use of Cuc-GUARD in greenhouse cucumber crops.

4.3 Incident reports related to the environment

Attenuated CGMMV strain ON-BM3; Al-Bio10 is a new active ingredient pending registration for use in Canada, and as of 15 March 2022, no incident reports had been submitted to the PMRA.

5.0 Value

Cucumber green mottle mosaic virus was first discovered in Canada in 2013 and is rapidly spreading in greenhouse cucumber production areas. Currently, the principal means to mitigate the risk of infection by aggressive CGMMV are procedures to thoroughly clean and disinfect the greenhouse after each crop and the use of CGMMV-free seed and transplants. Cuc-GUARD is the first pest control product for use to manage CGMMV.

Value information generated in greenhouse studies demonstrated that Cuc-GUARD applied at 4 to 8 L product/ha as a high pressure spray is effective in protecting cucumber plants against aggressive CGMMV. Cucumber seedlings treated with Cuc-GUARD were more vigorous and either did not present characteristic symptoms of CGMMV or symptoms were only slightly detectable. Cuc-GUARD treatment resulted in a significant increase in the proportion of cucumber fruit in the highest marketable grade while significantly reducing unmarketable fruit. This shift in fruit grade was more pronounced at the higher rate of 8 L product/ha. When applied in accordance with use directions, Cuc-GUARD can be expected to protect cucumber plants from aggressive CGMMV.

A CGMMV management strategy is a priority for Canadian greenhouse cucumber growers due to the significant threat this virus poses to the production of this high value crop. Cuc-GUARD will serve as the first in-crop option to manage this economically important disease.

6.0 Pest control product policy considerations

6.1 Toxic Substances Management Policy considerations

The Toxic Substances Management Policy (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, in other words, those that meet all four criteria outlined in the policy: 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*. The *Pest Control Products Act* requires that the TSMP be given effect in evaluating the risks of a product.

During the review process, Attenuated CGMMV strain ON-BM3; Al-Bio10 Technical and Cuc-GUARD were assessed in accordance with the PMRA Regulatory Directive DIR99-03[9] and evaluated against the Track 1 criteria. The PMRA has reached the conclusion that Attenuated CGMMV strain ON-BM3; Al-Bio10 Technical and Cuc-GUARD do not meet the Track 1 criteria because the active ingredient is a biological organism and hence is not subject to the criteria used to define persistence, bioaccumulation and toxicity properties of chemical control products.

6.2 Formulants and Contaminants of Health or Environmental Concern

During the review process, contaminants in the technical as well as formulants and contaminants in the end-use product are compared against Parts 1 and 3 of the *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern*[10]. The list is used as described in the PMRA Notice of Intent NOI2005-01[11] and is based on existing policies and regulations, including the Toxic Substances Management Policy and Formulants Policy[12] and taking into consideration the *Ozone-depleting Substance Regulations, 1998* under the *Canadian Environmental Protection Act* (substances designated under the Montreal Protocol). The PMRA has reached the following conclusions:

- Technical grade product Attenuated CGMMV strain ON-BM3; Al-Bio10 Technical and the end-use product, Cuc-GUARD, do not contain any formulants or contaminants identified in the *List of Pest Control Product Formulants of Health or Environmental Concern*.

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

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

10 SI/2005-114, last amended on 25 June 2008. See Justice Laws website, *Consolidated Regulations, List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern*.

11 PMRA's Notice of Intent NOI2005-01, *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern under the New Pest Control Product Act.*

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

7.0 Proposed Regulatory Decision

Health Canada's PMRA, under the authority of the *Pest Control Products Act*, is proposing registration for the sale and use of Attenuated CGMMV strain ON-BM3; Al-Bio10 Technical and Cuc-GUARD, containing the technical grade active ingredient attenuated CGMMV strain ON-BM3; Al-Bio10, to protect cucumber plants from virulent forms of CGMMV.

An evaluation of available scientific information found that, under the approved conditions of use, the health and environmental risks and the value of the pest control products are acceptable.

List of abbreviations

°C	degree(s) Celsius
cm	centimetre
cm^3	centimetres cubed
ARMS	amplification refractory mutation system
CGMMV	*Cucumber green mottle mosaic virus*
g	gram
ha	hectare(s)
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
L	litres
mL	millilitre
MPCA	microbial pest control agent
MRL	maximum residue limit
NIOSH	National Institute for Occupational Safety & Health
OECD	Organisation for Economic Co-operation and Development
PMRA	Pest Management Regulatory Agency
PPE	personal protective equipment
RNA	ribonucleic acid
RQ	risk quotient
RT-PCR	reverse transcription polymerase chain reaction
TSMP	Toxic Substances Management Policy

Appendix I Tables and figures

Table 1 List of supported uses

Crop	Greenhouse cucumber
Pest	*Cucumber green mottle mosaic virus* (CGMMV)
Application rate	4-8 L product/ha
Spray Volume	160-300 L/ha
Application method	high pressure spray (552-827 kPa) using a spray robot typically used in greenhouse cucumber production or with portable greenhouse sprayers
Application timing	when cucumber seedlings are between 2- to 3-weeks old
Number of applications	one per crop cycle
Other instructions	Seedlings to be treated with Cuc-GUARD should be grown from CGMMV-free seed, otherwise, plant samples must be submitted to a laboratory for analysis to confirm CGMMV-free status prior to treatment with Cuc-GUARD.

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