Pest Management Regulatory Agency
Agence de réglementation de la lutte antiparasitaire

Regulatory Note REG2006-02

Bacillus sphaericus Strain 2362

The biopesticide active ingredient *Bacillus sphaericus* strain 2362 and associated end-use products VectoLex WSP, VectoLex CG and VectoLex WDG containing *Bacillus sphaericus* serotype 5a5b, strain 2362 for the control of mosquito larvae in various aquatic habitats have been granted temporary registrations under the Pest Control Products Regulations.

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

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Foreword

Health Canada's Pest Management Regulatory Agency (PMRA) has issued temporary registrations for the microbial biopesticide *Bacillus sphaericus* strain 2362 and associated end-use products VectoLex WSP, VectoLex CG and VectoLex WDG for the control of mosquito larvae in various aquatic habitats.

The PMRA has carried out an assessment of available information in accordance with the Pest Control Products Regulations and has found it sufficient to allow a determination of safety, merit and value. The Agency has concluded that the use of the microbial biopesticide *Bacillus sphaericus* strain 2362 and associated end-use products VectoLex WSP, VectoLex CG and VectoLex WDG for the control of mosquito larvae in various aquatic habitats has merit and value consistent with the Pest Control Products Regulations and does not entail an unacceptable risk of harm. Therefore, based on the considerations outlined above, the microbial biopesticide *Bacillus sphaericus* strain 2362 and associated end-use products VectoLex WSP, VectoLex CG and VectoLex WDG have been granted temporary registration under the Pest Control Products Regulations, subject to the generation of confirmatory data.

Microbial pest control agents are increasingly being investigated for use as alternatives to conventional pesticides because they are thought to pose a lower potential risk to human health and the environment compared with conventional pesticides. VectoLex WSP, VectoLex CG and VectoLex WDG represent a potential biological replacement for chemical pesticides.

Valent BioSciences Corporation will be carrying out confirmatory studies as a condition of this temporary registration. Following the review of this information, the PMRA will publish a proposed registration decision document and request comments from interested parties before proceeding with a final regulatory decision.

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1.0 The Active Substances, its Properties and Uses

1.1 Identity

Identification of the Technical Grade Active Ingredient

Active micro-organism	*Bacillus sphaericus* strain 2362
Function	Mosquito larvicide
Binomial name	*Bacillus sphaericus*
Taxonomic designation	
Kingdom	Eubacteria
Phylum	Firmicutes
Class	Bacilli
Order	Bacilliales
Family	Bacillaceae
Genus	*Bacillus*
Species	*sphaericus*
Serovar	5a5b
Strain	2362
Patent Status Information	None
Nominal purity of active ingredient	670 *Bacillus sphaericus* International Toxic Units (BsITU) per milligram
Identity of relevant impurities of toxicological, environmental and/or other significance	The technical grade of the active ingredient, VectoLex Technical Powder, 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 (MPCA), *Bacillus sphaericus* strain 2632.

1.2 Physical and Chemical Properties

Technical Product: VectoLex Technical Powder

Property	Result
Colour	Tan (brown, grey-brown)
Odour	Musty
Physical state	Powder
Density/bulk density/specific gravity	0.52 g/mL
Viscosity	Not applicable
Corrosion character (oxidizing or reducing action)	Not reported (none expected)
Wettability	Not reported
pH (10% aqueous slurry)	6.3
Moisture content	Not reported

End-use Products: VectoLex WDG, VectoLex CG and VectoLex WSP

Property	Result		
	VectoLex WDG	**VectoLex CG**	**VectoLex WSP[a]**
Colour	Brown	Tan to brown	Tan to brown
Odour	Musty/acidic	Characteristic grainy	Characteristic grainy
Physical state	Fine granules	Granules	Granules
Formulation type	Water dispersable granules	Granular	Granular
Guarantee	650 BsITU/mg	50 BsITU/mg	50 BsITU/mg
Formulants	These products do not contain any USEPA List 1 formulants or formulants known to be TSMP Track 1 substances.		
Container material and description	5 kg plastic container or in larger bags	18 kg bags	Water soluble pouches (40/package)

Property	Result		
	VectoLex WDG	VectoLex CG	VectoLex WSP[a]
Corrosion character (oxidizing or reducing action)	Not reported (none expected)	Not reported (none expected)	Not reported (none expected)
Wettability	3 seconds	Not reported	Not reported
Density/bulk density/specific gravity	0.36 g/mL	0.52 g/mL	0.52 g/mL
pH (10% aqueous slurry)	5.01	6.3	6.3
Moisture content	Not reported	Not reported	Not reported
Storage stability	Storage stability studies indicated that these products were stable at 25°C for a period of up to 12 months.		

[a] VectoLex WSP has the same formulation as VectoLex CG, but is packaged in water soluble pouches.

1.3 Details of Uses and Further Information

VectoLex WDG, VectoLex CG and VectoLex WSP are end-use products containing the active ingredient *Bacillus sphaericus* strain 2362.

VectoLex WDG (650 BsITU/mg) is a water dispersible granule formulation proposed under Use-site Category 2, Aquatic Non-food Sites, as a biological mosquito larvicide to control mosquito larvae in various aquatic habitats: freshwater marshes, salt marshes, flood plains, flooded fields and pastures, wetlands, ponds, storm water detention/retention and seepage ponds, wastewater sewage effluent, sewage lagoons, oxidation ponds, log ponds, impounded waste water, septic ditches, drainage ditches including open storm sewers and irrigation ditches. VectoLex WDG is proposed to be applied as an aqueous suspension, using ground and aerial application equipment. The proposed aquatic sites are to be treated when mosquito larvae are known to be present.

VectoLex CG (50 BsITU/mg) is a corncob-based granule formulation proposed under Use-site Category 2, Aquatic Non-food Sites, as a biological mosquito larvicide to control mosquito larvae in various aquatic habitats: freshwater marshes, salt marshes, flood plains, flooded fields and pastures, wetlands, ponds, storm water detention/retention and seepage ponds, wastewater sewage effluent, sewage lagoons, oxidation ponds, log ponds, impounded waste water, septic ditches, drainage ditches including open storm sewers, irrigation ditches and waste tires. VectoLex CG is proposed to be applied, without dilution or mixing, to the water surface by hand, or by ground or aerial application methods. The proposed aquatic sites are to be treated when mosquito larvae are known to be present.

VectoLex WSP (50 BsITU/mg) is a water soluble pouch (10 g VectoLex CG/pouch) proposed under Use-site Category 2, Aquatic Non-food Sites, as a biological larvicide to control mosquito larvae in storm water catch basins. VectoLex WSP is proposed to be applied directly to catch basins without dilution. Catch basins are to be treated when mosquito larvae are known to be present.

Bacillus sphaericus strain 2362 was isolated in 1981 in Nigeria by Dr. J. Weiser (Institute of Entomology, Academy of Sciences of the Czech Republic) from an adult blackfly (*Simulium damnosum*). The strain was deposited at the Institut Pasteur, where it was housed in the World Health Organization Collaborating Centre and made available to interested scientists. Abbott Laboratories obtained the strain from Dr. A.A. Yousten (Virginia Polytechnical Institute, Blacksburg, Virginia) in 1982. Strain 2362 was identified as *Bacillus sphaericus* by cellular fatty acid analysis. Flagellar antigen analysis included it within the 5a5b serotype group. The strain could be uniquely identified by its antibiotic resistance profile and from the morphology of its crystalline inclusions (toxin proteins) produced during sporulation. *Bacillus sphaericus* is an aerobic endospore-forming bacterium belonging to the family Bacilliaceae. Both insecticidal and non-entomopathogenic strains can be found worldwide in water, soil and insects.

In *Bacillus sphaericus*, insecticidal activity is attributed to two distinct toxin types: the mosquitocidal toxin (Mtx) and the binary toxin (Btx). Mtx is a mosquitocidal adenosine diphosphate (ADP)-ribosylating toxin produced during vegetative growth, but its biological activity is insufficient to provide commercially relevant mosquito control. Btx contributes the primary toxic activity of commercial insecticidal strains. Btx is composed of two proteins, designated P51 and P42 based on their predicted molecular weights, which are associated with the spore as a toxin crystal. It is toxic by ingestion to mosquito larvae. After ingestion, the protein crystal matrix is dissolved in the anterior stomach. Midgut proteinases and high pH slowly convert P42 to a 39 kDa active form, and rapidly cleave P51 to a 43 kDa active form. P42 appears to be the active (toxic) component, and P51 contributes binding specificity. In susceptible species, midgut alterations begin as soon as 15 minutes after ingestion, especially in the posterior stomach and gastric cecae. In *Culex pipiens*, vacuolization of midgut cells is observed in electron micrographs, whereas *Anopheles stephensi* shows areas of low electron density. Mitochondrial swelling is observed in all susceptible species, and mitochondrial uptake and/or choline acetyltransferase may be inhibited. Late damage to neural tissue and skeletal muscle are also reported.

2.0 Methods of Analysis

2.1 Methods for Analysis of the Micro-organism as Manufactured

2.1.1 Methods for Identification of the Micro-organism

Bacillus sphaericus is an aerobic endospore-forming bacterium belonging to the family Bacillaceae. The vegetative cell is a Gram-positive rod, and the round, phase-bright terminal endospore significantly distends the cell wall. Although spore morphology is often used to identify *Bacillus sphaericus* microscopically, eight other *Bacillus* species produce similar spores. Phenotypic traits defining the species are few and include general or negatively defined traits such as the presence of sphaerical spores, the inability to grow anaerobically and the inability to metabolize sugars as carbon sources. However, DNA hybridization studies, flagellar serology and phage typing can differentiate insecticidal from saprophytic strains. Five strain groups were identified based on DNA homology. Mosquito pathogenic strains cluster in homology group IIA and fall into two groups, according to level of toxicity. Highly toxic strains, including the MPCA, produce a parasporal inclusion or toxin protein crystal, similar to that produced by *Bacillus thuringiensis*. In strains of lower toxicity, Mtx produced during vegetative growth is thought to confer toxicity. *Bacillus sphaericus* strains are also classified according to serotype. Strains of group IIA belonging to serotypes H25, H5a and 5b are the most toxic. The MPCA is of serotype H-5a5b. Phage typing identifies seven distinct lysotypes within DNA homology group IIA. The most toxic strains, including the MPCA, cluster in lysotype 3. Finally, in SDS-PAGE enzyme analysis, amino peptidase migration is strain specific and can be used in conjunction with phagotypy and serotypy for strain identification. In addition to methods described in the submitted literature, 16S rDNA sequencing, cellular fatty acid analysis to distinguish field or clinical isolates from commercial insecticidal strains, and random amplification of polymorphic DNA (RAPD) techniques for strain differentiation have been described in the published literature.

2.1.2 Methods for Establishment of Purity of Seed Stock

Lyophilized “cell banks” prepared from the original culture are maintained under vacuum in sealed glass ampules. Sufficient material was generated to last hundreds of years. From a single lyophilized ampule, a “master stock” is prepared. The cell bank tube is tested for vacuum, and the lyophilized culture is resuspended in a flask of liquid medium. The flask is incubated, transferred to vials containing cryoprotectant and stored at -80°C. Gram stains are prepared from selected vials, and cells are grown in broth and on agar to confirm culture purity. The master stock is generated in sufficient quantity to last 10 to 20 years. A “working stock” is generated in the same manner from the master stocks in sufficient quantity to last two to five years. Culture purity testing is performed on the residue in the master stock vial and in the growth flask. Production inoculum has, thus, undergone only four passages prior to production of the biopesticide product. At each passage, the new batch is tested against the current production stock. The selected vials are thawed; the cells are then observed microscopically (Gram staining), grown in broth

and streaked on agar to check culture purity. Each new working batch is tested in a pilot plant, then a production plant to ensure that fermentation kinetics and yield are similar to the current production batch.

2.1.3 Methods to Define the Content of the Micro-organism in the Manufactured Material Used for the Production of Formulated Products

A dipteran bioassay is used to determine potency. In the potency bioassay, the quantal dose response of two-day-old *Culex quinquefaciatus* larvae is analysed by weighted probit log dose regression and expressed in BsITU/mg according to Abbott's *Bacillus sphaericus* reference standard (Code I.D. 15385, Lot: 86-958-BD). A minimum of four replicates per sample is prepared on sequential days. Three preparations of a reference standard are run on each day of the assay. The assay is run in 100-mL paper cups, each containing 20 two-day-old *Culex quinquefaciatus* larvae. Six doses are tested for each sample, with three cups per dose. The test substance is suspended in water. The volume of the test substance is calculated based on the predicted potency and the volume of reference standard used. In some cases, 0.2% Tween 80 is added to aid resuspension. A 1/50 000 or 1/100 000 dilution of the test substance is prepared. This is further diluted tenfold, then five more times by 55% at each dilution in the paper cups. The cups are incubated at 28°C, 50% relative humidity, with a 12h/12h light cycle. After 42–45 hours, the number of live and dead larvae in each cup are counted. Probit analysis software is used to calculate and compare the median lethal dose (LD_{50}) of the sample and standard as well as to convert these to a relative potency expressed in BsITU/mg.

While the 86-958-BD reference standard has been used since the VectoLex products were first developed, it was assigned a different potency (3000 BsITU/mg) prior to 1995 than it is currently assigned (1700 BsITU/mg). The reference potency of 3000 BsITU/mg was used in bioassays submitted to secure registration with the United States Environmental Protection Agency (USEPA) in 1991. However, for business reasons, VectoLex products were not commercialized immediately following registration. When the product line was reactivated in 1995, Abbott recalibrated the 86-958-BD standard against Institut Pasteur standards and changed its assigned biopotency to 1700 BsITU/mg. Submitted potency data generated prior to 1995 were calculated according to the 3000 BsITU/mg standard, and those generated after 1995 according to the 1700 BsITU/mg standard. For this reason, potency determinations obtained in older studies may not correspond to those currently guaranteed on the label.

Potency data for five production batches were submitted and found to be acceptable.

2.1.4 Methods for the Determination of Relevant Impurities in the Manufactured Material

VectoLex Technical Powder is routinely tested for microbial contamination. Standard tests include total aerobic microbial count, total yeast count and mould count as well as testing for Enterobacteriaceae, coliforms and *Escherichia coli, Pseudomonas aeruginosa, Clostridium perfringens, Staphylococcus aureus* and *Salmonella* spp. Total aerobic counts are on tryptic soy agar pour or spread plates, incubated at 30–35°C. Alternatively, swab samples are suspended in Letheen broth (BD Diagnostics Systems) with 1% sodium metaphosphate, lactose broth, or Enterococcosel™ medium (BD Diagnostics Systems), then pour plated. Yeasts and moulds are counted on Sabouraud dextrose or potato dextrose pour plates. Alternatively, samples may be filtered through a 0.45 µm filter, and the filter plated.

Enterobacteriaceae are isolated on M-enterococcus agar pour plates on which they form pink to maroon colonies and microscopically appear as Gram-positive cells in chains. Presumptive colonies are identified by API® strip (bioMérieux) or VITEK® (bioMérieux) biochemical analysis.

Coliforms and *Escherichia coli* are detected on violet-red bile agar pour plates with an overlay. Coliforms form red-purple colonies, are Gram-negative and oxidase positive. If coliforms are present, API® strip or VITEK® biochemical profile analysis is conducted to confirm culture identity. To detect *Pseudomonas aeruginosa,* centrimide agar spread plates are incubated at 35–37°C and read 48–72 hours after inoculation. Bluish-green colonies are counted and their identity confirmed by Gram stain (negative), oxidase test and/or API® strips.

Clostridium perfringens is isolated on Oxoid perfringens agar pour plates, cultured anaerobically for 18–24 hours at 35–37°C. Large black colonies are indicative of *Clostridium perfringens* contamination. Presumptive colonies are confirmed by stab culture in nitrate motility agar (cells are non-motile, production of red colour indicates nitrate production) and in lactose gelatin tubes (*Clostridium perfringens* can liquefy gelatin).

Staphylococcus aureus is detected on Baird-Parker or egg yolk tellurite agar. Black shiny colonies surrounded by a clear zone are presumtive for *Staphylococcus aureus*. Gram stain and coagulase tests confirm culture identity.

To detect *Salmonella* species, the samples are first enriched in lactose broth, then grown in selective media, selenite cystine broth or fluid tetrathionate, before plating on brilliant green agar. Small pink or white colonies, which may be surrounded by a red zone, are indicative of *Salmonella*. Alternatively, xylose lysine desoxycholate agar are used, on which red colonies with or without a black centre are presumptive of *Salmonella,* or bismuth sulfite agar, productive of black or dark green *Salmonella* colonies. Confirmation of culture identity is with a triple sugar agar stab/slant and API® analysis.

Maximum allowable limits for contaminating micro-organisms are < 1000 organisms/g for enterococci/streptococci and < 100 organisms/g for coliforms. Data from five production batches of each end-use product were submitted, and microbial contamination was within acceptable limits for each.

2.1.5 Methods to Show Absence of Any Human and Mammalian Pathogens

In addition to microbial screening for potential pathogens described in Section 2.1.4, a mouse safety test confirms the absence of *Bacillus anthracis* in the slurry for each batch. Detailed protocols were submitted. These were identical to those used for quality control of registered *Bacillus thuringiensis* pesticides.

2.1.6 Methods to Determine Storage Stability, Shelf-life of the Micro-organism

A single lot (86-958-BD) of VectoLex Technical Powder was tested for potency after 12 and 24 months of storage at 25°C. Initial potency was 3000 BsITU/mg; at 12 months, potency was 2172 BsITU/mg (a 28% loss); and, at 24 months, potency was 2122 (a 29% loss). Although the loss in potency on storage is significant, the applicant claims that potency estimation of the technical grade active ingredient will be done immediately prior to formulation. This is acceptable as long as the proportions of ingredients in the formulation remain within the range specified on the Statement of Product Specification.

The short-term stability of potency in one lot (22-515-BD) of VectoLex Technical Powder was assessed under various physical conditions. Storage temperature did not appear to affect the potency of the powder for up to 48 hours. No decrease in potency was observed after 24 or 48 hours at 5°C or 50°C. Exposure to dry air for 1, 2 and 4 weeks at 25°C did not appear to affect potency. Where testing required suspension of the technical grade product in water (for testing the effect of pH and metal ions on potency), microbial growth became excessive at 48 hours. Exposure of a suspension of VectoLex Technical Powder to metal ions [0.01 M $MgCl_2$, NaCl, $CuCl_2$, $FeCl_2$, $Pb(C_2H_3O_2)_3$ or $SbCl_4$] did not affect potency. In aqueous suspension, all tested samples lost potency between 24 and 48 hours, including the untreated control; this may have been due to microbial contamination of the test samples. Potency was most significantly affected by pH when in suspension. The suspension was stable for 48 hours at pH 5, but a 24% loss in potency was observed at 24 hours at pH 7, and a 67% loss in potency was observed by 24 hours at pH 9. This might be expected, as the alkaline pH in the insect midgut is known to solubilize the parasporal crystal. Finally, exposure of VectoLex Technical Powder to simulated sunlight significantly reduced potency at 96 hours.

Submitted storage stability data indicated that VectoLex WDG, VectoLex CG and VectoLex WSP are stable for a period of 12 months when stored in the original packaging at 25°C.

3.0 Impact on Human and Animal Health

See Appendix I, Table 1, for a summary table.

3.1 Integrated Toxicity and Infectivity Summary

The acute toxicity and infectivity studies submitted to support the registration of VectoLex Technical Powder, VectoLex WDG, VectoLex CG and VectoLex WSP included numerous acute oral, acute dermal, acute pulmonary/inhalation, injection infectivity, dermal irritation and eye irritation studies. Many of these studies were classified as acceptable, while others were classified as supplemental. VectoLex Technical Powder was not overtly toxic or pathogenic to the rat via the oral route, was not overtly toxic or pathogenic to the rat via the pulmonary route and was not pathogenic to the rat via the intravenous route. VectoLex Technical Powder did cause significant irritation (moderate to severe erythema and barely perceptible edema) in the acute dermal toxicity study; however, there were no signs of toxicity. In irritation studies, VectoLex Technical Powder was slightly irritating to the skin and mildly irritating to the eyes of rabbits. VectoLex WDG was not toxic to rats via the oral and inhalation routes, and was not toxic to rabbits via the dermal route. VectoLex WDG was slightly irritating to the skin of rabbits and minimally irritating to the eyes of this same species. For VectoLex CG and VectoLex WSP, Tier I acute mammalian testing was waived because the toxicological and irritation properties of these products were not expected to be greater than VectoLex Technical Powder. These products were, however, considered to be mild ocular irritants given that all micro-organisms are considered to be irritating to eyes in the absence of data. VectoLex Technical Powder and the end-use formulations are also considered to be a potential sensitizers as all micro-organisms, including *Bacillus sphaericus* strain 2362, are considered to contain substances that would elicit a positive allergic reaction in test animals.

As noted in Section 1.3, the insecticidal activity of *Bacillus sphaericus* strain 2362 is attributed to two distinct toxin types: Mtx and Btx. Mtx is of lower insecticidal activity and is produced only during vegetative growth. Btx is the commercially important mosquitocidal toxin expressed during sporulation and accumulated as a protein protoxin crystal within the exosporangium of the spore. Mtx is an ADP-ribosyl-transferase toxin with broad-spectrum activity that shares sequence homology with several known bacterial toxins that are active against eukaryotic cells, including Diphtheria toxin, *Pseudomonas* exotoxin A, Cholera toxin and Pertussis toxin. As such, it poses a potential human-health concern. However, the mature Mtx protein is bound to the membrane of the vegetative cell (protoxin) and must be processed by chymotrypsin or mosquito gut extract to yield the active toxin. Furthermore, the Mtx protoxin is degraded by intracellular proteases during sporulation. As such, in a production batch of *Bacillus sphaericus* strain 2362 grown to stationary phase (to permit sporulation and formation of Btx, the commercially relevant toxin), little Mtx toxin is expected to remain. Although the absence of Mtx in VectoLex formulations was not demonstrated in the submission, the toxin is known to be further inactivated during processing, thus leaving little or no

active Mtx in end-use formulations. Mtx is expressed only in vegetative cells. After release of the product, vegetative cells are expected to occur only within the host insect when ingested spores have germinated. Therefore, the potential for human contact with Mtx is expected to be low.

With respect to potential effects on the endocrine system, there are no reports or indications in the available scientific literature that suggest *Bacillus sphaericus* strain 2362 in VectoLex WDG, VectoLex CG and VectoLex WSP has caused, or has the potential to cause, adverse effects on the endocrine system of animals. Also, there are no reports that would implicate *Bacillus sphaericus* strain 2632 as a potential producer of genotoxins.

None of the formulants used in VectoLex Technical Powder, VectoLex WDG, VectoLex CG and VectoLex WSP are of toxicological concern.

3.2 Reporting of Hypersensitivity Incidence

A dermal sensitization study was submitted instead of reporting the incidence of hypersensitivity with *Bacillus sphaericus* strain 2362 on researchers, handlers and applicators. Sensitization data are not normally required for microbial pest control agents as these studies are generally limited to dermal exposures and do not usually address other routes of exposure (e.g., pulmonary). Also, most micro-organisms contain substances that would elicit a positive response in test animals. Therefore, all formulations containing microbial pest control agents are considered potential sensitizing agents. Continued surveillance and reporting of hypersensitivity incidents are required.

3.3 Impact on Human and Animal Health Arising from Exposure to the Active Substance or to Impurities Contained in it

3.3.1 Occupational and Bystander Exposure Assessment

The potential occupational and bystander exposures resulting from the proposed uses of VectoLex WDG, VectoLex CG and VectoLex WSP are expected to vary significantly between products. The greatest variation is expected to occur between VectoLex WSP and the two other proposed end-use formulations, VectoLex CG and VectoLex WDG. VectoLex WSP is identical to the VectoLex CG formulation, but it is contained within water soluble pouches and is only intended to control *Culex* and *Culiseta* mosquito larvae in storm water catch basins. VectoLex CG is intended to control *Culex* mosquito larvae as well as species of *Culiseta* and *Aedes*. This product is to be directly applied to freshwater marshes, salt marshes, flood plains, flooded fields and pastures, wetlands, ponds, storm water detention/retention and seepage ponds, wastewater sewage effluent, sewage lagoons, oxidation ponds, log ponds, impounded waste water, septic ditches as well as drainage ditches including open storm sewers and irrigation ditches using conventional ground or aerial application equipment. This product is also to be applied by hand to individual waste tires. The proposed use of VectoLex WDG is similar to VectoLex CG. However, VectoLex WDG must be diluted prior to application to

freshwater marshes, salt marshes, flood plains, flooded fields and pastures, wetlands, ponds, storm water detention/retention and seepage ponds, wastewater sewage effluent, sewage lagoons, oxidation ponds, log ponds, impounded waste water, septic ditches as well as drainage ditches including open storm sewers and irrigation ditches using conventional ground or aerial application equipment.

When handled according to label instructions, minimal occupational and bystander exposure is expected from the proposed use of VectoLex WSP. As previously noted, VectoLex WSP is contained within a water soluble pouch; therefore, little exposure is expected during application. The proposed use sites also limit exposure as these are usually inaccessible to the general public. The potential for occupational and bystander exposure is much greater for VectoLex CG and VectoLex WDG, as both products are to be broadcast over larger areas using conventional ground or aerial application equipment (fixed wing or helicopter equipped with either conventional boom or rotary atomizer). Vectolex CG is also to be applied by hand into individual waste tires. VectoLex WDG and VectoLex CG are to be used similarly with the exception that VectoLex WDG is to be diluted in water prior to application. For VectoLex CG, exposure from mixing, loading and clean-up activities is expected to be primarily via the dermal route; however, inhalation, oral and ocular exposure may also occur from fine particles that may be present in the end-use formulation. For VectoLex WDG, mixer and loader exposure is expected to be primarily via the dermal route; however, inhalation, oral and ocular exposure may also occur from fine particles that may be present in the end-use formulation. Clean-up exposure, however, is expected to be mostly via the dermal route since the granular formulation is suspended in water prior to application. During application, exposure to both products is expected to be primarily via the dermal and inhalation routes, although ocular and oral exposure can also occur. Bystander exposure is also possible near application areas; however, this exposure should be minimized if VectoLex CG and VectoLex WDG are applied under the appropriate meteorological conditions.

As no significant toxicity and no pathogenicity was observed in Tier I acute mammalian studies, it is recommended that all three end-use product labels include standard personal protective equipment (long-sleeved shirt, long pants and shoes plus socks). The labels for VectoLex WDG and VectoLex CG must also include waterproof gloves and a MSH/NIOSH-approved dust/mist filtering respirator with approval number prefix TC-21C or a NIOSH-approved respirator with any –95, R-95, P-95 or HE filter. A respirator is required to reduce the possibility of workers developing allergies or other types of hypersensitive reactions following repeated inhalation exposure to *Bacillus sphaericus* strain 2632. The wearing of a respirator would also prevent the possible gradual accumulation of *Bacillus sphaericus* spores in the lungs after repeated exposures, as long clearance times were reported in acute pulmonary studies with VectoLex Technical Powder. Eye goggles are also required on the label for VectoLex CG as this

product was considered to be a mild ocular irritant. The requirements for eye goggles and a respirator on the VectoLex WSP label were waived based on the limited potential for exposure when handling intact water soluble pouches. The integrity of the water soluble pouches may be compromised if they are allowed to become wet; the pouches must be kept dry.

4.0 Residues

4.1 Residue Summary

Bacillus sphaericus strain 2362 will not to be directly applied to food/feed or to treated, finished drinking water. However, there is a possibility that live *Bacillus sphaericus* spores may be found on crops sown in treated fields or fields irrigated with treated water. The *Bacillus sphaericus* spores may also be found in drinking water as it is not known if municipal water treatment processes will destroy these spores. No adverse effects are expected from this exposure based on the lack of adverse effects noted in the mammalian toxicity and infectivity studies. Therefore, the establishment of a maximum residue limit is not required for *Bacillus sphaericus* under the *Food and Drugs Act* and Regulations.

5.0 Fate and Behaviour in the Environment

Environmental fate data (Tier II/III) were not required, as significant adverse effects to most non-target organisms were not expected from the proposed use of the MPCA. Although a higher tier ecotoxicology study has been requested, this does not in itself trigger environmental fate testing requirements at this time.

5.1 Expected Environmental Concentration

The expected environmental concentration (EEC) of the MPCA after a single application to water was calculated to be 1.1 mg/L immediately following application, assuming a water depth of 15 cm (as specified in Regulatory Directive DIR2001-02, *Guidelines for the Registration of Microbial Pest Control Agents and Products*) and a scenario in which the maximum label rate for VectoLex WDG of 1.68 kg/ha is applied once. VectoLex WDG was used for calculation of the EEC because its potency (650 BsITU/mg) approaches that of VectoLex Technical Powder (670 BsITU/mg).

It is anticipated that VectoLex products will be applied more than once per season. Calculation of a repeated-application EEC requires the input of a variable to represent the persistence of the active ingredient: usually a half-life (assuming first-order decay kinetics). The concept of a half-life is not meaningful for a live organism, which may replicate in the environment. In an attempt to define the persistence of the MPCA, the published literature on its environmental fate was considered. In an investigation of the persistence of *Bacillus sphaericus* strain 2362 spores in the aquatic environment (Yousten et al. 1992), water samples were inoculated with the MPCA and incubated under different environmental conditions. Spores were recovered at a series of timepoints

thereafter. Recovered water samples were heated to 80°C for 15 minutes before plating on NYSM agar. The plates were incubated at 30°C for 48 hours before colonies were counted. Note that the heat treatment used during spore recovery kills vegetative cells, so it is impossible to differentiate a decline in spore count due to cell death from a decline in count due to spore germination. In the laboratory, in a sample of filtered pond water (dissolved organic carbon [DOC] 1.5 mg/L), a 30% decrease in heat-resistant spores was seen after 238 days. In unfiltered pond water, containing a finely-divided sediment of rotting leaves and other organic matter (DOC 158 mg/L), there was a 64% decline in heat-resistant spore count after 35 days, a 90% decline after 63 days and a 92% decline after 238 days. However, in a subsequent experiment, the heat-resistant spore count was done in conjunction with a direct microscopic count using a vital dye (acridine orange) that detects viable, vegetative cells. Although a 97.5% decline in the heat-resistant spore number was recorded after 49 days, the direct (microscopic) count did not decline. The authors did not comment on the significance of this difference, but it suggests that the declining spore count could be due to germination and outgrowth of vegetative cells, rather than to death of the spores. If this were the case, the organism could not be assumed to disappear from the aquatic environment at the rates suggested in this study. Therefore, this study was considered to be unacceptable.

A conservative EEC was calculated using a very high half-life value of 10 000 days (over 27 years). Although *Bacillus sphaericus* strain 2362 spores are vulnerable to certain physical and biological elements in the environment, they may accumulate in the benthic layer of aquatic habitats, protected from harmful UV radiation and sunlight. The phenomenon of “recycling” (propagation of spores in mosquito larvae) and possible replication in nutrient-rich environments may also sustain spore numbers for an extended period. The VectoLex product labels stipulate a minimum interval of seven days between applications, but place no restriction on the total number of applications. For the purposes of this calculation, a maximum 20-week season of application is assumed, with weekly applications at the maximum label rate for VectoLex WDG of 1.68 kg/L, to a 15 cm depth of water. This EEC, representing the maximum conceivable single-season accumulation of the MPCA, was 22.3 mg/L.

6.0 Effects on Non-target Species

6.1 Effects on Terrestrial Organisms

Submitted terrestrial environmental effect studies suggest that *Bacillus sphaericus* is of low toxicity to birds. The potential for harm in mammalian wildlife species was addressed in infectivity and toxicity studies on laboratory mammals. These studies indicated that there is no significant toxicity to rodents from acute oral testing at the maximum hazard dose. The risk to mammalian wildlife is expected to be minimal. Submitted studies also suggest that *Bacillus sphaericus* is of low toxicity to honey bees. Although non-target testing of insects other than honey bees could only be considered as supplementary data, honey bee testing is sufficient to meet the data requirement for non-target terrestrial arthropod testing. Supplemental bioassay data suggest that *Bacillus*

sphaericus is toxic to *Trichoplusia ni* and *Leptinotarsa decemlineata* at high dietary concentrations, and that the *Bacillus sphaericus* host range may be wider than previously understood. It is important that the arthropod host range of *Bacillus sphaericus* strain 2362 be well defined. For this reason, a replacement bioassay is required. The data requirement for non-arthropod invertebrate testing was waived. In an extensive search of the literature, only a patent for the use of a *Bacillus sphaericus* spore extract to inhibit hatching of nematode eggs suggested any potential for non-target effects in non-arthropod invertebrates. *Bacillus sphaericus* is not thought to be pathogenic to nematodes. Soil micro-organism effects data were not required for VectoLex products as they are not intended to control pest micro-organisms, and the biology, ecology and proposed use pattern indicate little potential for adverse effects in soil microbes. The requirement for terrestrial plant testing was waived based on the narrow host range of *Bacillus sphaericus* as an insect pathogen. *Bacillus sphaericus* is not known as a plant pathogen, and no incidents of adverse effects in plants have been reported in over a decade of use in the United States, in sites including rice fields. Sufficient information and data were submitted to address terrestrial environmental effects, except in defining the arthropod host range of the MPCA. As noted above, a replacement bioassay is required.

See Appendix II, Table 1, for a summary table of effects on terrestrial organisms.

6.2 Effects on Aquatic Organisms

Submitted aquatic environmental effect studies suggest that *Bacillus sphaericus* strain 2362 is practically non-toxic to aquatic birds (mallard ducks), freshwater and marine fishes, chironomid larvae, mysid shrimp and unicellular algae. *Bacillus sphaericus* strain 2362 was slightly toxic to the aquatic cladoceran *Daphnia magna*. The 31-day EC_{50} for daphnid survival was 2.82×10^{10} CFU/L, and the no observed effect concentration (NOEC) for growth and reproductive effects was 4.86×10^{9} CFU/L. These concentrations are greater than those anticipated to occur, even with repeated applications of VectoLex over an extended season at the highest application rate permitted on the label.

In the course of aquatic and marine fish and invertebrate testing, it was observed that the addition of *Bacillus sphaericus* strain 2362 to test waters caused a marked decline in the dissolved oxygen concentration, with subsequent clinical and behavioural signs of hypoxia in test organisms (such as frequent surfacing in sheepshead minnow). This effect was highly concentration dependent and is not expected to occur at the application rates permitted on the product label. Furthermore, many treated sites, especially those that provide habitats for aquatic and marine non-target organisms, are expected to be readily re-aerated by the action of wind and waves.

The potential for ecologically significant hypoxia in treated water bodies is considered to be low. Shell deposition in *Crassostrea virginica* (Eastern oyster) was significantly impaired in oysters exposed to high concentrations of *Bacillus sphaericus* strain 2362. This effect did not appear to be related to hypoxia. The 96-hour EC_{50} for shell deposition

was 42 mg/L (or approximately 2×10^6 CFU/L). Oysters exposed to test concentrations at or above the EC_{50} did not feed, and oysters exposed to 29 mg/L *Bacillus sphaericus* exhibited reduced feeding. The EEC for a 20-week season of weekly applications at the maximum label rate would equal the NOEC for shell deposition in oysters if the half-life of *Bacillus sphaericus* strain 2362 in the aquatic environment was 106 days. Due to the nature of the MPCA as a live organism, a meaningful half-life cannot be established, but persistence of the MPCA for 106 days is conceivable and even likely. A longer-term toxicity/infectivity study in Eastern oyster or in a susceptible Canadian freshwater bivalve mollusc is, therefore, required. A study from the published literature (Lacey and Mulla 1990), which focused on aquatic arthropods that are predaceous on or share habitats with mosquito larvae, indicated that there is little risk to non-target aquatic arthropods from *Bacillus sphaericus* strain 2362 applied at mosquito larvicidal rates. Sufficient information and data were submitted to address aquatic environmental effects, except with respect to toxicity in molluscs. As noted above, a long-term study in Eastern oyster or a Canadian freshwater bivalve mollusc is required.

See Appendix II, Table 2, for a summary table of effects on aquatic organisms.

7.0 Efficacy

7.1 Effectiveness of VectoLex WSP, VectoLex CG and VectoLex WDG Against Target Organisms

7.1.1 Intended Use

Valent Biosciences Ltd. has applied for restricted class registrations of three end-use products containing *Bacillus sphaericus* strain 2362. The three end-use products are VectoLex WSP Biological Larvicide, containing 50 BsITU/mg (10 g pouch); VectoLex CG Biological Larvicide, containing 50 BsITU/mg; and VectoLex WDG Biological Larvicide, containing 650 BsITU/mg. All three end-use products intend to be used for the control of mosquito larvae in Use-site Category 2, Aquatic Non-food Sites. See the following tables for detailed intended uses of the three proposed products.

Proposed Label Claims of VectoLex WSP

Pest controlled	Larvae of mosquito species susceptible to *Bacillus sphaericus* including *Culex pipiens, Culex restuans, Culiseta incidens* and *Culiseta inornata*
Use sites	Storm water catch basins
Dosages	One pouch (10 g) per catch basin
Timing of application	Apply to the mosquito breeding sites where mosquito larvae are known to be present
Reapplication interval	Reapply as needed after 1 to 4 weeks
Application methods	Apply the pouch in the basins by hand

Proposed Label Claims of VectoLex CG

Pest controlled: Larvae of the listed mosquito species	*Culex* spp., *Aedes vexans, Aedes cinereus, Aedes* (*Ochlerotatus*) *triseriatus, Aedes* (*Ochlerotatus*) *dorsalis, Aedes* (*Ochlerotatus*) *melanimon, Aedes* (*Ochlerotatus*) *nigromaculis, Aedes* (*Ochlerotatus*) *stimulans, Aedes* (*Ochlerotatus*) *trivittatus, Culiseta incidens, Culiseta inornata*
Use sites and dosages	**Water bodies:** 5.5–16.5 kg/ha—freshwater marshes, salt marshes, flood plains, flooded fields and pastures, wetlands, ponds, storm water detention/retention and seepage ponds, wastewater sewage effluent, sewage lagoons, oxidation ponds, log ponds, impounded waste water, septic ditches, drainage ditches including open storm sewers and irrigation ditches **Waste tires:** 0.376 g/tire only if application is made directly to each tire
Timing of application	Apply to the mosquito breeding sites where mosquito larvae are known to be present and treat larvae following egg hatch through the 4th instar larval stage
Reapplication interval	Reapply as needed after 1 to 4 weeks
Application methods	Applied directly without dilution by aerial or ground application equipment

Proposed Label Claims of VectoLex WDG

Pest controlled: Larvae of the listed mosquito species	*Culex* spp., *Aedes vexans, Aedes cinereus, Aedes* (*Ochlerotatus*) *triseriatus, Aedes* (*Ochlerotatus*) *dorsalis, Aedes* (*Ochlerotatus*) *melanimon, Aedes* (*Ochlerotatus*) *nigromaculis, Aedes* (*Ochlerotatus*) *stimulans, Aedes* (*Ochlerotatus*) *trivittatus, Culiseta incidens, Culiseta inornata*
Use sites and dosages	**Water bodies:** 0.55–1.7 kg/ha—freshwater marshes, salt marshes, flood plains, flooded fields and pastures, wetlands, ponds, storm water detention/retention and seepage ponds, wastewater sewage effluent, sewage lagoons, oxidation ponds, log ponds, impounded waste water, septic ditches, drainage ditches including open storm sewers and irrigation ditches
Timing of application	Apply to the mosquito breeding sites where mosquito larvae are known to be present and treat larvae following egg hatch through the 4th instar larval stage
Reapplication interval	Reapply as needed after 1 to 4 weeks
Application methods and water volume	Apply in water dilution Aerial (in 5–90 L of water) and ground (in 10–200 L of water)

7.1.2 Mode of Action

The active ingredient of VectoLex WSP, VectoLex CG and VectoLex WDG is *Bacillus sphaericus* strain 2362. *Bacillus sphaericus* strain 2362 is a spore-forming bacterium, toxic to many species of mosquito larvae upon ingestion. During sporulation, *Bacillus sphaericus* produces a crystal toxin composed of two proteins. After ingestion of the spore-crystal complex by mosquito larvae, the crystal matrix quickly dissolves and becomes activated in the lumen of the midgut through the combined action of midgut proteinases and the high pH of midgut. The release of toxins from crystals occurs in all species of mosquitoes, even in less susceptible species such as *Aedes aegypti*. The difference in susceptibility to *Bacillus sphaericus* between mosquito species depends on the presence of a specific receptor on the midgut membranes where the protein toxins bind. In less susceptible species, there seems to be fewer receptors on the midgut membrane. The toxins either do not bind to the midgut or rapidly leak out from midgut cells. In susceptible mosquito species, midgut damage starts as soon as 15 minutes after ingestion of the spore-crystal complex. The resulting damage to the midgut cells leads to death. Previous research has shown that *Culex* spp. are more susceptible to *Bacillus sphaericus,* while *Aedes* spp. are less susceptible to *Bacillus sphaericus*.

7.1.3 Use Sites

VectoLex WSP is proposed for use in storm water catch basins.

VectoLex CG is proposed for use in various water bodies—freshwater marshes, salt marshes, flood plains, flooded fields and pastures, wetlands, ponds, storm water detention/retention and seepage ponds, wastewater sewage effluent, sewage lagoons, oxidation ponds, log ponds, impounded waste water, septic ditches, drainage ditches including open storm sewers, irrigation ditches and waste tires (only if application is made directly to each tire).

VectoLex WDG is proposed for use in various water bodies—freshwater marshes, salt marshes, flood plains, flooded fields and pastures, wetlands, ponds, storm water detention/retention and seepage ponds, wastewater sewage effluent, sewage lagoons, oxidation ponds, log ponds, impounded waste water, septic ditches, drainage ditches including open storm sewers and irrigation ditches.

7.1.4 Effectiveness Against Pest

7.1.4.1 Effectiveness of VectoLex WSP

7.1.4.1.1 *Culex* spp. in Catch Basins

Five efficacy trials conducted in Canada (British Columbia, Ontario, Quebec) and the northern United States (Oregon, Michigan, Massachusetts, Illinois) assessed the efficacy of VectoLex WSP against the larvae of *Culex pipiens, Culex restuans* and *Culex stigmatosoma* in catch basins. The application rate tested was one pouch per basin, and the product was applied by hand. The catch basin size used in the trials ranged between 0.25 m^2 and 1 m^2. Water samples were taken using a mosquito larval dipper to determine the pretreatment pest pressure by counting the number of early instar larvae in the water samples. The post-treatment performance was assessed by counting the number of mosquito larvae and pupae in water samples taken at intervals of days or weeks post-treatment. The percentage of mosquito larval control was calculated by comparing the number of mosquito larvae and pupae in treated versus untreated and corrected by pretreatment larval count. The results indicated that 94–100% larval reduction was achieved in all the trials at one week post-treatment. The percentage of control after 1 week varied, and over 90% larval reduction lasted from 2 to 6 weeks post-treatment, depending on the trial. One trial demonstrated over 98% larval reduction on week 15 post-treatment. Considering the unpredictable nature of aquatic sites, the product can be reapplied when needed, as determined by monitoring for mosquito larvae after one week. The data showed that it normally took up to a week for the product to demonstrate 100% larval reduction.

In Canada, there are only four *Culex* species: *Culex pipiens, Culex restuans, Culex tarsalis* and *Culex territans*. All *Culex* species tested are susceptible to *Bacillus sphaericus* toxins. A control claim for *Culex* spp. is supported by the submitted efficacy data.

The proposed control claim for *Culex* spp. in catch basins at one pouch per basin is acceptable. The product should not be reapplied within one week of application and reapplications can be made at a minimum interval of one week if sampling indicates that further applications are required. VectoLex WSP should be applied at mosquito larval stage, preferably at younger larval stages.

7.1.4.1.2 *Culiseta* spp. in Catch Basins

Three efficacy trials conducted in British Columbia, Quebec and Oregon assessed the efficacy of VectoLex WSP against *Culiseta incidens* and *Culiseta inornata* in catch basins. Application rate tested was one pouch per basin, and the product was applied by hand targeting the larval stage. The results indicated that 100% larval reduction was achieved 1 week after application and > 90% larval reduction continued for up to 4 weeks post-treatment. Observations after 4 weeks post-treatment were not conducted on *Culiseta* spp. The data showed that it normally took up to a week for the product to demonstrate the 100% larval reduction.

In Canada, there are seven *Culiseta* species. *Culiseta incidens* and *Culiseta inornata* are the most abundant species and often occur in the same habitat as *Culex* spp. *Culiseta*, as a genus, has not been shown to be less susceptible to *Bacillus sphaericus* toxins than *Aedes* spp. A control claim for *Culiseta* spp. is supported by the submitted efficacy data and similarity in biology between the major Canadian *Culiseta* spp.

The proposed control claim for *Culiseta* spp. in catch basins at one pouch per basin is acceptable. The product should not be applied within one week of application and reapplications can be made at a minimum interval of one week if sampling indicates that further applications are required.

7.1.4.2 Effectiveness of VectoLex CG

7.1.4.2.1 *Culex* spp. in Various Water Bodies (except waste tires and catch basins)

Thirty-one efficacy trials conducted in Canada (British Columbia, Manitoba and Quebec) and the United States (Washington, Michigan, Oregon, Iowa, Connecticut, Pennsylvania, Utah, California and Texas) assessed the efficacy of VectoLex CG against *Culex pipiens, Culex tarsalis, Culex territans* and *Culex restuans* in various aquatic sites (artificial pools, ditches, flooded field, waste water pond, dairy waste pool, irrigated corn field, rice field, irrigated horse pastures, woodland pools, wetland, swamp, flood river, boat basin, marshland, sewage lagoons). The product was applied without dilution by ground application or aerial application methods. The application rates tested were between 2.2 and 22.4 kg/ha. The results indicated that > 90% larval reduction lasted for 1–7 weeks post-treatment at 5.6–16.8 kg/ha, depending on the trials. Two efficacy studies tested a rate below 5.6 kg/ha and showed inferior results. The data showed that in water with high level of suspended matter, the application rate of 16.8 kg/ha provided better control than the lower rates tested. The data showed that it normally took up to a week for the VectoLex WDG to demonstrate the 100% larval reduction.

The proposed control claim for *Culex* spp. is acceptable at 5.6–16.8 kg/ha, with the higher rate required in aquatic sites with high levels of suspended matter. The product should not be reapplied within one week of application and reapplications can be made at a minimum interval of one week if sampling indicates that further applications are required. VectoLex CG should be applied at larval stage, preferably targeting younger larval stages.

7.1.4.2.2 *Culiseta* spp. in Various Water Bodies (except waste tires and catch basins)

Four efficacy trials conducted in Manitoba, Oregon and Washington assessed the efficacy of VectoLex CG against *Culiseta incidens* and *Culiseta inornata* in pastures and artificial pools. The rate tested were between 2.3 and 16.5 kg/ha, and the results indicated that > 90% larval reduction lasted for 2–3 weeks post-treatment at 5.5–16.5 kg/ha. Post-treatment observations beyond three weeks were not conducted. One trial tested the rate of 2.3 kg/ha and showed inferior results to the higher rates tested.

The proposed control claim for *Culiseta* spp. is acceptable at 5.6–16.8 kg/ha, with the higher rate required in aquatic sites with a high levels of suspended matter.

7.1.4.2.3 *Aedes* spp. in Various Water Bodies (except waste tires and catch basins)

Aedes vexans: Four efficacy trials conducted in British Columbia and the United States (New Jersey, Washington and Michigan) assessed the efficacy of VectoLex CG against *Aedes vexans* in flooded rivers, sewage outfall, irrigated pasture and ditches. The product was applied by ground application targeting larval stages. The rates tested were between 5 and 16.8 kg/ha and the results indicated that 85–100% larval reduction lasted up to 5 days. Post-treatment observations beyond 5 days were not conducted in these trials.

Aedes* (*Ochlerotatus*) *melanimon: Two efficacy trials conducted in California assessed the efficacy of VectoLex CG against a mixed population of *Culex* spp. and *Aedes* (*Ochl.*) *melanimon* in flooded fallowfield and wetland. The product was applied by air. The result of one trial indicated 100% larval reduction for 17 days post-treatment at 5.6 kg/ha, compared to pretreatment population. No untreated check was included in this trial and whether the 100% control observed was due to treatment or natural population decline cannot be determined. The other trial had very low pest pressure and the result was questionable.

Aedes* (*Ochlerotatus*) *dorsalis: One efficacy trial conducted in Utah assessed the efficacy of VectoLex CG against *Aedes* (*Ochl.*) *dorsalis* in an irrigated pasture. The rate tested was 11.2 kg/ha and the product was applied by air. The result demonstrated 98% larval reduction for 14 days post-treatment compared to pretreatment. No untreated check was included in this trial and whether the 100% control observed was due to treatment or natural population decline cannot be determined.

Aedes* (*Ochlerotatus*) *nigromaculis: One efficacy trial conducted in California assessed the efficacy of VectoLex CG against *Aedes* (*Ochl.*) *nigromaculis* in an irrigated pasture. The rates tested were 2.8 and 5.6 kg/ha and the product was applied by ground application equipment. The result indicated that the two rates provided 91 and 98% larval reduction, respectively, on day 2 post-treatment. No further observations were conducted beyond 2 days post-treatment. The control level at one week post-treatment was unknown.

Aedes* (*Ochlerotatus*) *trivittatus: One efficacy trial conducted in Iowa assessed the efficacy of VectoLex CG against a mixed population of *Culex pipiens* and *Aedes* (*Ochl.*) *trivittatus* in ditches. The rates tested were 2.8 and 5.6 kg/ha and the product was applied by ground application equipment. The assessment was made on day 3 post-treatment. The result indicated that *Aedes* (*Ochl.*) *trivittatus* was not controlled by VectoLex CG using either of these two rates. However, *Culex pipiens* was effectively controlled at the same rates in this trial.

Aedes cinereus, Aedes* (*Ochlerotatus*) *triseriatus* and *Aedes* (*Ochlerotatus*) *stimulans: No efficacy studies were provided.

The submitted data support the control claim for *Aedes vexans*, but indicated that other *Aedes* and *Ochlerotatus* species have variable degrees of susceptibility to VectoLex CG. A control claim for *Aedes vexans* is supported. The statement that other *Aedes* and *Ochlerotatus* spp. have variable degrees of susceptibility to VectoLex CG should be included on the label.

7.1.4.2.4 *Culex* spp. and *Culiseta* spp. in Waste Tires

One efficacy trial conducted in Illinois assessed the efficacy of VectoLex CG against the larvae of *Aedes* (*Ochlerotatus*) *triseriatus, Culex restuans, Culex pipiens* and *Anopheles punctipennis* in waste tires. The product was applied by hand, at a rate of 0.376 g product/tire, to tires hung on a fence. The result demonstrated 100% larval reduction of *Aedes* (*Ochlerotatus*) *triseriatus, Culex restuans* and *Culex pipiens* for 25 days post-treatment compared to the untreated check. However, the treatment was not effective for the *Anopheles punctipennis* larvae. The proposed rate, 0.376 g/tire, may not be adequate for all types of tires, which can vary considerably in size. An appropriate rate expression is the amount of product/m^2 of water surface. The acceptable application rates for other aquatic habitats can be extrapolated to water inside individual tires.

The control claim for *Culex spp. Culiseta* spp. and *Aedes* (*Ochlerotatus*) *triseriatus* is acceptable at 0.56–1.68 g product/m^2 of water surface for waste tires, only if the product can be applied directly to individual tires. The higher rate is required for water with a high level of suspended matter. Use of a larvicide in waste tires is not the best way to control mosquitoes. The most effective way to control mosquitoes in this situation is to eliminate the standing water in waste tires. This can be achieved by shredding, removing, drilling holes in or covering the waste tires.

7.1.4.3 Effectiveness of VectoLex WDG

7.1.4.3.1 *Culex* spp. in Various Water Bodies (except catch basins and waste tires)

Twenty-nine efficacy trials conducted in British Columbia and United States (Washington, Michigan, Oregon, Pennsylvania, California and Texas) assessed the efficacy of VectoLex WDG against *Culex pipiens, Culex tarsalis, Culex quinquefasciatus, Culex solinarius* and *Culex nigripalpus* in various aquatic sites (artificial pools, rivers, flooded pastures, sewage lagoons, flooded duck club, irrigation ditches, wetland, waste water pond, flooded river, storm water retention area, septic ditches, woodland pools, rice fields and salt marshes). The product was applied in water dilutions by ground or aerial application equipment when mosquito larvae were present. The application rates tested were between 0.56 and 1.68 kg/ha. The results indicated > 90% larval reduction lasting for 2–4 weeks post-treatment, depending on the trial. The data demonstrated that in aquatic sites with high levels of suspended matter, the higher application rate was required. The data showed that it normally took up to a week for VectoLex WDG to demonstrate the 100% larval reduction.

The proposed control claim for *Culex* spp. is acceptable at 0.56–1.68 kg/ha, with the higher rate required in sites with high levels of suspended matter. The product should not be reapplied within one week of application and reapplications can be made at a minimum interval of one week if monitoring indicates that further applications are required. VectoLex WDG should be applied at larval stage, preferably targeting younger larval stages.

7.1.4.3.2 *Culiseta* spp. in Various Water Bodies (except waste tires and catch basins)

One efficacy trial conducted in Washington assessed the efficacy of VectoLex WDG against *Culiseta incidens* in artificial pools. The trial tested application rates of 0.056 and 0.112 kg/ha against a mixed population of *Culex* spp. and *Culiseta incidens*. The result indicated > 90% larval reduction lasting for 3 weeks post-treatment at 0.112 kg/ha. The rate of 0.056 kg/ha showed inferior performance. Even though only one trial was conducted with VectoLex WDG on *Culiseta* spp., more trials were conducted with VectoLex CG against *Culiseta* spp. Seven side-by-side trials comparing VectoLex CG and VectoLex WDG demonstrated biological equivalency of the two formulations. The control claim of VectoLex WDG for all species in the genus *Culiseta* is supported by the provided data and extrapolation from the submitted VectoLex CG data.

The proposed control claim for *Culiseta* spp., at rates of 0.56–1.68 kg/ha, with the higher rate required in sites with high levels of suspended matter is supported.

7.1.4.3.3 *Aedes* spp. in Various Water Bodies (except waste tires and catch basins)

Aedes vexans: One efficacy trial conducted in British Columbia assessed the efficacy of VectoLex CG against *Aedes vexans* in a flooded river. The product was applied by ground application equipment. The rate tested was 0.5 kg/ha, and the results demonstrated 94% larval reduction on day 2 post-treatment. No further observations were conducted beyond 2 days post-treatment.

Aedes (Ochlerotatus) nigromaculis: One efficacy trial conducted in Oregon assessed the efficacy of VectoLex WDG against *Aedes* (*Ochl*.) *nigromaculis* in flooded pasture. The rates tested were 0.56 and 1.12 kg/ha, and the product was applied by ground application equipment. The post-treatment performance was assessed until day 14. The result demonstrated > 90% larval reduction only on day 2 post-treatment at both rates.

Aedes cinereus, Aedes* (*Ochlerotatus*) *triseriatus Aedes* (*Ochl.*) *dorsalis, Aedes* (*Ochl.*) *melanimon, Aedes* (*Ochl.*) *stimulans* and *Aedes* (*Ochl.*) *trivittatus: No efficacy data were provided.

Very limited efficacy data on *Aedes* spp. were provided. The biological equivalency of VectoLex WDG and VectoLex CG were tested and established in seven side-by-side trials comparing the two formulations. The proposed control claim for *Aedes vexans,* with the statement that other *Aedes* and *Ochlerotatus* spp. have variable degrees of susceptibility to VectoLex WDG, is supported by extrapolation from the VectoLex CG data.

7.1.5 Total Spray Volume

VectoLex CG and VectoLex WSP are to be applied without any dilution or mixing. VectoLex WDG is to be applied in water. Three efficacy trials conducted with VectoLex WDG compared low and high water volumes: 28 and 56 L/ha, 56 and 112 L/ha by ground application, and 9.3 and 28 L by aerial application. The results indicated that the low or high water volume did not affect the efficacy of VectoLex WDG.

The proposed water volumes, 10–200 L of water by conventional ground application and 5–90 L by aerial application, are acceptable.

7.2 Phytotoxicity to Target Plants or Target Plant Products

No specific data of effects of *Bacillus sphaericus* on plants were submitted and no phytotoxicity studies were required. *Bacillus sphaericus* is a naturally occurring organism, known for its selectivity against mosquitoes. No adverse effects on flora or non-target fauna are expected. No adverse effects on plants have been observed in the United States since the VectoLex products were marketed in 1995 or in other countries around the world where *Bacillus sphaericus* is used for mosquito control.

7.3 Impact on Succeeding Crops, Adjacent Crops and on Treated Plants or Plant Products Used for Propagating

7.3.1 Impact on Adjacent Crops

No adverse effects on flora or non-target fauna are expected. No adverse effects on crops have been observed in the United States since the VectoLex products were marketed in 1995 or in other countries around the world where *Bacillus sphaericus* is used for mosquito control.

7.3.2 Tank Mixing Recommendations

VectoLex WSP, VectoLex CG and VectoLex WDG are proposed to be used alone and not to be tank mixed with other products.

7.4 Economics

Mosquito control as a public health measure is aimed at controlling mosquito populations known to transmit human diseases. In Canada, much of the current effort in mosquito control is to curb the establishment and impact of West Nile virus. West Nile virus is spread by mosquitoes that have fed on the blood of infected birds. A number of mosquito species, such as *Culex pipiens*, known to carry West Nile virus, have been demonstrated to be susceptible to *Bacillus sphaericus*. Furthermore, it is anticipated that the use of VectoLex products will further reduce the reliance on chemical larvicides for mosquito control in sensitive aquatic habitats.

7.5 Sustainability

7.5.1 Survey of Alternatives

Mosquito larvae can be controlled by non-larvicidal means or by larvicidal means. Non-larvicidal means including water management and source reduction (improved drainage, filling and levelling low lying areas, improved sanitation, habitat modification and removal of potential mosquito larval habitats) are the most effective and economical means of providing long-term mosquito control. Natural enemies (predators and diseases) are also important in reducing mosquito larval populations. Where non-larvicidal means are ineffective or impractical, mosquito larvicides will be used to control mosquito populations.

The major alternative mosquito larvicides, including chemical and biological, currently registered for use in Canada include, but are not necessarily limited to the following:

Alternative Mosquito Larvicide

Technical Grade Active Ingredient	End-use Products		Insecticide Classification		Site	Application Rate (a.i./ha)
	Name	Class	Group	Mode of Action		
Malathion	Fyfanon 50% EC	C	1B	Contact, stomach and respiratory	Standing water	550 g/ha
	Malathion 500 E	C	1B		Standing water	550 g/ha
Malathion	Gardex Malathion ULV Concentrate	C	1B	Contact, stomach and respiratory	Mosquito larval breeding area	404–522.5 mL/ha
	Wilson Malathion EC	C	1B		Fish and farm ponds, or dugouts	0.55 L/ha
	Wilson Malathion ULV Insecticide Concentrate	C+R	1B		Mosquito larval breeding area	261–404 mL/ha
Diflubenzuron	Dimilin	R	15	Growth regulator	Non-crop areas containing temporary pools	27.5–47.5 g/ha
Chloropyrifos	Dursban Turf Insecticide	C	1B	Contact, stomach and respiratory	Temporary pools (e.g., shallow, grassy depression, flooded woodlands, industrial parks, roadway ditches, railway marshalling yards, small temporary sloughs).	13–53 g /ha
	Dursban 2 ½ G	R	1B		Temporary pools. Not for use in permanent water bodies such lakes, dugout or fish ponds	28–46.25 g/ha
	Dursban T	C	1B		Temporary pools. Not for use in permanent water bodies such lakes, dugout or fish ponds	13–106 g/ha
Methoprene	Altosid XR Briquets	R	7B	Growth regulator	Storm sewers and catch basins	1–4 briquet (0.756–3.024 g a.i./basin)
	Altosid Pellets	R	7B		Floodwater Permanent water	0.119–0.476 kg/ha 0.238–0.476 kg/ha
	Altosid Granules	R	7B		Floodwater Standing water	84–168 g/ha 168–336 g/ha

Technical Grade Active Ingredient	End-use Products		Insecticide Classification		Site	Application Rate (a.i./ha)
	Name	Class	Group	Mode of Action		
Bacillus thuringiensis israelensis	Aquabac II *xt*	R	11A1	Stomach poison	Flood water, roadside ditches, irrigation ditches, pools in woodlands, snow melt pools. Tidal water, salt marshes, catch basins, storm water retention areas. Polluted water (sewage lagoons, etc.)	0.384–0.768 billion ITU/ha 0.76–1.536 billion ITU/ha 1.536–3.072 billion ITU/ha
	Aquabac II *xt*	C	11A1		Flood water, roadside ditches, irrigation ditches, pools in woodlands, snow melt pools. Tidal water, salt marshes, catch basins, storm water retention areas. Polluted water (sewage lagoons, etc.)	0.384–0.768 billion ITU/ha 0.768–1.536 billion ITU/ha 1.536–3.072 billion ITU/ha
Bacillus thuringiensis israelensis	Aquabac (200G) Granules (5/8)	C	11A1	Stomach poison	Flood water, roadside ditches, irrigation ditches, pools in woodlands, snow melt pools, tidal water, salt marshes, catch basins, storm water retention areas.	0.5–4 billion ITU/ha
	AquaBac (200G) Granules (10/14)	D	11A1		Standing waters, wholly contained on the homeowner property.	1 billion ITU/ha
	VectoBac 200G	C	11A1		Standing water: Temporary and permanent pools in pastures and woodlots, irrigation or roadside ditches, natural marshes or estuarine areas, waters contiguous to fish-bearing water, catch basins and sewage lagoons.	0.6–2 billion ITU/ha
	VectoBac 200G	R	11A1		Same as above	Same as above
	VectoBac 1200L Aqueous Suspension	R	11A1		Temporary pools in pastures and woodlots, irrigation or roadside ditches, natural marshes or estuarine areas, catch basins and sewage lagoons.	0.3–1.2 billion ITU/ha

Technical Grade Active Ingredient	End-use Products		Insecticide Classification		Site	Application Rate (a.i./ha)
	Name	Class	Group	Mode of Action		
	Teknar® Granules	R	11A1		Flood water, pastures, standing ponds and ditches. Tidal water and salt marshes, catch basins and storm water retention areas. Water polluted with sewage, water with moderate organic content and water with a high level of suspended solids	1.17–1.742 billion AA units/ha 1.456–1.742 billion AA units/ha 1.742 billion AA units/ha
	Teknar® HP-D	R	11A1		Floodwaters, pastures, standing ponds and ditches. Tidal water and salt marshes, catch basins and storm water retention areas. Water polluted with sewage, water with moderate organic content and water with high level of suspended solids.	0.9–1.8 billion AA units/ha 1.8–3.6 billion AA units/ha 3.6 billion AA units/ha

7.5.2 Compatibility with Current Management Practices Including Integrated Pest Management

VectoLex WSP, VectoLex CG and VectoLex WDG are compatible with current mosquito management practices and have the potential to further reduce or replace the use of chemical larvicides in aquatic habitats. The toxins of *Bacillus sphaericus* are known for their host selectivity and are expected to have minimal effect on natural enemies of mosquitoes.

Current mosquito control programs include larval monitoring, which is necessary to ensure that VectoLex WSP, VectoLex CG and VectoLex WDG are applied to target the susceptible stages of mosquitoes for maximum effectiveness.

7.5.3 Contribution to Risk Reduction

It is expected that VectoLex WSP, VectoLex CG and VectoLex WDG will be used in various aquatic habitats for mosquito larval control, especially in sensitive aquatic habitats. VectoLex WSP, VectoLex CG and VectoLex WDG are most effective in controlling *Culex* spp. and will provide an alternative to *Bacillus thuringiensis* subspecies *israelensis* (Bti) based products, which are less effective against *Culex* spp. VectoLex WSP, VectoLex CG and VectoLex WDG have been demonstrated to be more effective and provide longer residual activity than Bti-based products in sites with high levels of suspended matter. The VectoLex products may reduce the need for chemical larvicide

applications, therefore, reducing the associated risks of resistance to the pesticide, of effects on workers and to the environment.

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

Mosquito larvae have been known to develop resistance to chemical larvicides. *Bacillus sphaericus*, like Bti, is a stomach poison and has a different mode of action from chemical larvicides. Evidence suggests that *Bacillus sphaericus* toxins have different mode of action than Bti toxins. Thus, VectoLex WSP, VectoLex CG and VectoLex WDG will provide an alternative biological control tool. Mosquito larvae have demonstrated resistance to *Bacillus sphaericus* in tropical countries, but the resistance has not been demonstrated in North America. To delay or avoid the development of resistance, the applicant has incorporated resistance management recommendations on the label. These recommendations are in line with PMRA Regulatory Directive DIR99-06, *Voluntary Pesticide Resistance Management Labelling Based on Target Site/Mode of Action*. The following resistance management signage is also recommended on the primary panel of the label:

GROUP	11A2	INSECTICIDE

7.6 Conclusions

VectoLex WSP
Efficacy data support the use of VectoLex WSP at the proposed application rate, one pouch per basin, for the control of larvae of *Culex* and *Culiseta* spp. in catch basins. The product can be reapplied at a minimum interval of one week if sampling indicates that further applications are required.

Vectolex CG
Efficacy data support the use of VectoLex CG at the proposed application rates, 5.6–16.8 kg product/ha, for control of the larvae of *Culex* spp., *Culiseta* spp. and *Aedes vexans* in various proposed aquatic habitats. Other *Aedes* and *Ochlerotatus* spp. showed variable degrees of susceptibility to VectoLex CG. The efficacy data support the use of VectoLex CG in waste tires only if the product can be applied to individual tires. The acceptable rates are 0.56–1.68 g product/m^2 of water surface. The data demonstrated that the higher rate was required in water with a high level of suspended matter. The product can be reapplied at a minimum interval of one week if sampling indicates that further applications are required.

VectoLex WDG

Efficacy data support the use of VectoLex WDG at the proposed application rates, 0.56–1.68 kg product/ha, for control of the larvae of *Culex* spp., *Culiseta* spp. and *Aedes vexans* in various proposed aquatic habitats. Other *Aedes* and *Ochlerotatus* spp. showed variable degrees of susceptibility to VectoLex WDG. The data demonstrated that the higher rate was required in aquatic sites with high levels of suspended matter. The product can be reapplied at a minimum interval of one week if sampling indicates that further applications are required.

7.6.1 Summary

Summary of Label Proposals and Recommendations of VectoLex WSP

Directions for Use	Proposed	Recommendation (based on value assessment)	Comments
Application Timing	Apply at mosquito larval stage	Same	With clarification on the label as follows: "Apply to catch basins when sampling indicates that mosquito larvae are present."
Number of Applications and Reapplication interval	No limit. Reapply after 1–4 weeks	No limit Reapply at a minimum interval of one week	Data showed that residual activity lasted for 2–15 weeks post-treatment.
Application Method	By hand	Same	With clarification on the label as follows: "Apply by hand and place the pouch in the centre of the catch basin."
Sites	Storm water catch basins	Same	
Mosquito Species	*Culex pipiens*, *Culex restuans*, *Culiseta incidens* and *Culiseta innornata*	*Culex* spp. and *Culiseta* spp.	A control claim including the whole two genera of *Culex* and *Culiseta* is supported by the data and the mosquito biology.
Spray Volume	—	—	Not applicable. Product is applied without dilution.

Summary of Label Proposals and Recommendations of VectoLex CG

Directions for Use	Proposed	Recommendation (based on value assessment)	Comments
Application Timing	Apply at mosquito larval stage	Same	With clarification on the label as follows: "Apply to mosquito breeding sites when sampling indicates that mosquito larvae are present."
Number of Applications and Reapplication Interval	No limit. Reapply after 1–4 weeks	No limit Reapply at a minimum one week interval	Data showed that residual activity lasted for 1–7 weeks.
Application Method	By ground and aerial	Same	Data support both application methods. Waste tires: apply by hand to individual tires.
Sites	**Water bodies** 5.6–16.8 kg/ha—freshwater marshes, salt marshes, flood plains, flooded fields and pastures, wetlands, ponds, storm water detention/retention and seepage ponds, wastewater sewage effluent, sewage lagoons, oxidation ponds, log ponds, impounded waste water, septic ditches, drainage ditches including open storm sewers and irrigation ditches	Freshwater marshes, salt marshes, flood plains, flooded field and pastures, wetlands, ponds, storm water detention/retention and seepage ponds, wastewater sewage effluent, sewage lagoons, oxidation ponds, log ponds, impounded waste water, septic ditches, drainage ditches including open storm sewers and irrigation ditches	Submitted data demonstrated that the rates of 5.6–16.8 kg/ha provided control of mosquito larvae in the aquatic sites tested. The higher application rate is required in water polluted with sewage, water with high organic content and water with a high level of suspended solids.
	Waste tires 0.376 g/tire—only if application is made directly to each tire	Waste tires only if the product can be applied to individual tires.	The data support the rates of 0.56–1.68 g product/m^2 of water surface. Use the higher rate in water with high organic content and water with a high level of suspended solids.

Directions for Use	Proposed	Recommendation (based on value assessment)	Comments
Mosquito Species	*Culex* spp., *Aedes vexans, Aedes cinereus, Aedes* (*Ochlerotatus*) *triseriatus, Aedes* (*Ochlerotatus*) *dorsalis, Aedes* (*Ochlerotatus*) *melanimon, Aedes* (*Ochlerotatus*) *nigromaculis, Aedes* (*Ochlerotatus*) *stimulans Aedes* (*Ochlerotatus*) *trivittatus, Culiseta incidens, Culiseta inornata*	*Culex* spp. *Culiseta* spp. *Aedes vexans* (Other *Aedes* and *Ochlerotatus* spp. have variable degrees of susceptibility to VectoLex CG)	Adequate efficacy data demonstrated to accept the control claim of *Culex* spp., *Culiseta* spp., *Aedes vexans* and other *Aedes* and *Ochlerotatus* spp., which have variable degrees of susceptibility to VectoLex CG.
Spray Volume	—	—	Not applicable. Product applied without dilution

Summary of Label Proposals and Recommendations of VectoLex WDG

Directions for Use	Proposed	Recommendation (based on value assessment)	Comments
Application Timing	Apply at mosquito larval stage	Same	With clarification on the label as follows: "Apply to mosquito breeding sites when sampling indicates that mosquito larvae are present."
Number of Applications and Reapplication Interval	No limit. Reapply after 1–4 weeks	No limit Reapply at a minium one week interval	Data showed that residual activity lasted for 1–7 weeks.
Application Method	By ground and aerial	Same	Data support both application methods.
Sites	**Water bodies** 0.55–1.7 kg/ha—freshwater marshes, salt marshes, flood plains, flooded fields and pastures, wetlands, ponds, storm water detention/retention and seepage ponds, wastewater sewage effluent, sewage lagoons, oxidation ponds, log ponds, impounded waste water, septic ditches, drainage ditches including open storm sewers and irrigation ditches	Same	Submitted data demonstrated that the rates of 0.56–1.68 kg/ha provided control of mosquito larvae in the aquatic sites tested. Use the higher rate in water polluted with sewage, water with high organic content and water with a high level of suspended solids.

Directions for Use	Proposed	Recommendation (based on value assessment)	Comments
Mosquito Species	*Culex* spp., *Aedes vexans, Aedes cinereus, Aedes* (*Ochlerotatus*) *triseriatus, Aedes* (*Ochlerotatus*) *dorsalis, Aedes* (*Ochlerotatus*) *melanimon, Aedes* (*Ochlerotatus*) *nigromaculis, Aedes* (*Ochlerotatus*) *stimulans Aedes* (*Ochlerotatus) trivittatus, Culiseta incidens, Culiseta inornata*	*Culex* spp. *Culiseta* spp. *Aedes vexans* (Other *Aedes* and *Ochlerotatus* spp. have variable degrees of susceptibility to VectoLex WDG)	Submitted data support the control claim of *Culex* spp., *Culiseta* spp., *Aedes vexans* and other *Aedes* and *Ochlerotatus* spp., which have variable degrees of susceptibility to VectoLex WDG.
Spray Volume	**Ground application** Apply the product in 10–200 L of water **Aerial application** Apply in 5–90 L of final spray mixture	Same	With clarification on the label as follows: "See Directions for Use section for specific use directions."

8.0 Toxic Substances Management Policy

Bacillus sphaericus strain 2362 in VectoLex WDG, VectoLex CG and VectoLex WSP does not meet TSMP 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. There are also no formulants, contaminants or impurities present in the end-use products that would meet the TSMP Track 1 criteria.

9.0 Regulatory Decision

The PMRA has carried out an assessment of available information in accordance with the Pest Control Products Regulations and has found it sufficient to allow a determination of safety, merit and value. The Agency has concluded that the use of the microbial biopesticide *Bacillus sphaericus* strain 2362 and associated end-use products VectoLex WSP, VectoLex CG and VectoLex WDG for the control of mosquito larvae in various aquatic habitats has merit and value consistent with the Pest Control Products Regulations and does not entail an unacceptable risk of harm. Therefore, based on the considerations outlined above, the microbial biopesticide *Bacillus sphaericus* strain 2362 and associated end-use products VectoLex WSP, VectoLex CG and VectoLex WDG have been granted temporary registration under the Pest Control Products Regulations, subject to the generation of following confirmatory data.

- A bioassay for lepidopterans and coleopterans
- A toxicity/infectivity study in a bivalve mollusc

List of Abbreviations

a.i.	active ingredient
BsITU	*Bacillus sphaericus* International Toxic Unit
Bti	*Bacillus thuringiensis israelensis*
Btx	binary toxin
bw	body weight
C	commercial
CD	cesarean derived
CFU	colony forming unit
CG	corn cob granules
CI	confidence interval
D	domestic
DOC	dissolved organic carbon
DNA	deoxyribonucleic acid
EC_{50}	median effective concentration
GSD	geometric standard deviation
h	hour(s)
ha	hectare
kDa	kilodalton
kg	kilogram
KTS	killed test substance
KTSI	killed test substance, immunosuppressed host
L	litre
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LOAEL	lowest observed adverse effect level
MAS	maximum average score
MCC	maximum challenge concentration
mg	milligram
mL	millilitre
MIS	maximum irritation score
MMAD	mean mass aerodynamic diameter
MPCA	microbial pest control agent
MSH	Mine Safety and Health Administration
Mtx	mosquitocidal toxin
NIOSH	National Institute of Occupational Safety and Health
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
PMRA	Pest Management Regulatory Agency
R	restricted
RAPD	random amplification of polymorphic DNA
rDNA	ribosomal DNA
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
TGAI	technical grade of the active ingredient
TS	live test substance

TSI	live test substance, immunosuppressed host
USC	Use-site Category
USEPA	United States Environmental Protection Agency
WDG	water dispersable granules
WSP	water soluble pouches

Appendix I Toxicology

Table 1 Summary of Toxicity and Infectivity Studies with VectoLex Technical Powder, VectoLex WDG, VectoLex CG and VectoLex WSP

STUDY	SPECIES, STRAIN AND DOSES	LD_{50}, NOAEL AND LOAEL	TARGET ORGAN, SIGNIFICANT EFFECTS, COMMENTS
ACUTE STUDIES			
VectoLex Technical Powder			
Oral toxicity/ pathogenicity	Rat—CD – 7/sex treated with TGAI (TS) in distilled water, 5 g/kg bw or ~2.8×10^{10} CFU/kg bw (measured), gavage – 7/sex treated with heat-killed TGAI (KTS), 5 g/kg bw, gavage	LD_{50} greater than 5 g/kg bw or 2.8×10^{10} CFU/kg bw	– 1/7 TS and 1/7 KTS females were found dead on Day 2. These two females had a torn esophagus and tan fluid in the thoracic cavity at necropsy. These deaths were attributed to gavage error. – 1/7 TS female was found dead on Day 6. This female was hypoactive from Day 1 to the time of death on Day 6, and also displayed a urine-wet abdomen. At necropsy, clear, dark and/or red contents were observed in the thoracic cavity, stomach, cecum, and small and large intestines, but no esophageal punctures were observed. – 1/7 KTS male had an enlarged spleen at interim sacrifice on Day 7. This was considered to be a normal immunological reaction. – Colonies were recovered from all tissues, urine and feces collected from TS and KTS animals. These results were questionable as the recovery medium used in the clearance assays was not selective and thus could not differentiate contaminating microbes in non-sterile tissues such as urine and feces. Sampling was also arbitrary and variable. Recovery counts were obtained from samples that were collected using a disposable sterile inoculating loop rather than homogenizing the sample and plating a known volume. The thermal inactivation procedure also did not destroy the spores even though this method should be sufficient to destroy this micro-organism. No pattern of clearance could be established. SUPPLEMENTAL because no pattern of microbial clearance was established.

STUDY	SPECIES, STRAIN AND DOSES	LD_{50}, NOAEL AND LOAEL	TARGET ORGAN, SIGNIFICANT EFFECTS, COMMENTS
ACUTE STUDIES			
Oral toxicity/ pathogenicity	Rat—Sprague Dawley – 15/sex treated with TGAI in saline, ~3×10^8 CFU/animal (measured), gavage – 15/sex treated with saline	LD_{50} greater than 3×10^8 CFU/animal	– No mortalities – No signs of toxicity – No lesions at necropsy – Colonies of *Bacillus sphaericus* were recovered from feces and lungs of treated animals. Isolation from lungs is likely due to dosing error or accidental aspiration of gavage material. LOW TOXICITY, NOT PATHOGENIC ACCEPTABLE
Inhalation toxicity/ pathogenicity	Rat—CD – 5/sex treated with TGAI (nominal: 4×10^{10} CFU/g), 4 hours, whole-body – 0.09 mg/L, MMAD = 8631 μm, GSD = 432	LC_{50} greater than 0.09 mg/L or 3.6×10^6 CFU/L	– No mortalities – No signs of toxicity – At necropsy, 1/5 male rats had multiple red foci on the lungs. 1/5 male rats had diffuse red discolourations on the lungs. These reactions are likely due to normal immunological reactions to foreign antigens such as spores of *Bacillus sphaericus*. – Sporadic and inconsistent recoveries of *Bacillus sphaericus* were reported. No distinct pattern of microbial clearance was established. SUPPLEMENTAL because no pattern of microbial clearance was established and the dose was very low.
Pulmonary toxicity/ pathogenicity	Rat—Sprague Dawley – 17/sex treated with TGAI in saline, 5.6×10^8 CFU/animal (measured) or 0.04 mL/animal, intratracheal – 17/sex treated with saline, 0.04 mL/animal, intratracheal	LD_{50} greater than 5.6×10^8 CFU/animal	– No mortalities – No signs of toxicity – Lung discolorourations (mottling, foci) were noted in 10/17 males and 12/17 females. – Colonies of *Bacillus sphaericus* were recovered from the lungs, liver and spleen. A pattern of microbial clearance was established by Day 49. LOW TOXICITY, NOT PATHOGENIC ACCEPTABLE

STUDY	SPECIES, STRAIN AND DOSES	LD_{50}, NOAEL AND LOAEL	TARGET ORGAN, SIGNIFICANT EFFECTS, COMMENTS
ACUTE STUDIES			
Injection (IV) infectivity	Mouse—CD1, newly-weaned – 20/sex treated with TGAI in saline (TS), 6×10^7 CFU/animal (measured) or 0.05 mL – 20/sex treated with TGAI in saline, 6×10^7 CFU/animal (measured) or 0.05 mL, immunosuppressed with cortisone acetate (TSI) – 5/sex treated with heat-killed TGAI in saline (KTS), 0.05 mL – 5/sex treated with heat-killed TGAI in saline, 0.05 mL, immunosuppressed with cortisone acetate (KTSI)	LD_{50} greater than 6×10^7 CFU/animal	– 7/20 TS males and 3/20 TS females died on Days 0–4. – 1/5 KTS male and 1/5 KTS female died on Day 1. – 1/5 KTSI female died on Day 1. – Clinical signs included hypoactivity (17/40 TS, 7/40 TSI, 3/10 KTS, 4/10 KTSI), laboured respiration (3/40 TS), urine-wet abdomen (2/40 TS, 1/40 TSI), pale (1/40 TS, 1/40 TSI), ataxic (3/40 TS), head tilt (1/40 TS), circling (1/40 TS), diarrhea (2/40 TS, 1/10 KTSI), prostration (1/40 TS), convulsion (1/40 TS), tail dark (4/40 TS, 3/40 TSI), distal portion of tail missing (3/40 TS, 3/40 TSI), and loss of use of hind leg (1/40 TS). The tail effects were considered to be related to dosing trauma in tail vein. – Necropsy findings included small spleen (1/40 TSI), enlarged spleen (13/40 TS, 18/40 TSI), dark gelatinous contents in intestines (1/40 TS), pale lungs (1/40 TSI), dark gelatinous substance on surface of brain (1/40 TS) and enlarged uterine horns (½0 TS, 2/20 TSI,1/5 KTS). – The timepoint when the clinical and necropsy findings were made was not reported nor was the affected mouse identified. These findings could be attributed to dead or moribund mice. – Colonies of *Bacillus sphaericus* were recovered from the blood, brain, lungs, liver and spleen of mice treated with test substance (TS, TSI). No distinct pattern of microbial clearance was established. SUPPLEMENTAL because no pattern of microbial clearance was established.

STUDY	SPECIES, STRAIN AND DOSES	LD_{50}, NOAEL AND LOAEL	TARGET ORGAN, SIGNIFICANT EFFECTS, COMMENTS
ACUTE STUDIES			
Injection (IV) infectivity	Hamster, newly-weaned – 20/sex treated with TGAI in saline (TS), 5×10^7 CFU/animal (measured) or 0.05 mL – 20/sex treated with TGAI in saline, 5×10^7 CFU/animal (measured) or 0.05 mL, immunosuppressed with cortisone acetate (TSI) – 5/sex treated with heat-killed TGAI in saline (KTS), 0.05 mL – 5/sex treated with heat-killed TGAI in saline, 0.05 mL, immunosuppressed with cortisone acetate (KTSI)	LD_{50} greater than 5×10^7 CFU/animal	– ½0 TSI females exhibiting characteristics of a spontaneous disease process in hamsters was found dead on Day 22. This mortality was not considered to be related to treatment. – The female that died on Day 22 exhibited lacrimation, salivation, hypoactivity, urine-wet abdomen, white exudate surrounding the eyes, and emaciation. – ½0 TSI females had exophthalmos (abnormal protrusion of eye), drying of the surface of one eye and hypoactivity. – On Day 28, ½0 TS females had hypothermia, was prostrate, and had laboured respiration. These effects were likely due to a transiently low room temperature in the morning. – 3/40 TSI animals lost weight (numbers not provided) – At necopsy, an apparent loss of structure in the right eye was noted in one TSI female. – Colonies of *Bacillus sphaericus* were recovered from the blood, brain, lungs, liver and spleen of mice treated with test substance (TS, TSI). No distinct pattern of microbial clearance was established. SUPPLEMENTAL because no pattern of microbial clearance was established.
Injection (IV) infectivity	Rat—Sprague Dawley, young adult – 17/sex treated with TGAI in saline, 1.4×10^7 CFU/animal (measured) or 0.2 mL – 17/sex treated with saline, 0.2 mL	LD_{50} greater than 1.4×10^7 CFU/animal	– No mortalities – No clinical signs – At necropsy, lung discolourations were noted in one male rat. – Colonies of *Bacillus sphaericus* were recovered from liver, lungs, spleen, kidney, blood, lymph nodes and brain of treated animals. Significant reductions in CFUs were recorded on Day 49. NOT PATHOGENIC ACCEPTABLE

STUDY	SPECIES, STRAIN AND DOSES	LD_{50}, NOAEL AND LOAEL	TARGET ORGAN, SIGNIFICANT EFFECTS, COMMENTS
ACUTE STUDIES			
Dermal toxicity	Rabbit—New Zealand white – 5/sex treated with TGAI at 2000 mg/kg bw or 2.4×10^{10} CFU/kg bw (measured) in 2 mL/g saline to at least 10% of the body surface, occluded for 24 hours, then wiped (not washed) – 5/sex untreated	LD_{50} greater than 2000 mg/kg bw or 2.4×10^{10} CFU/kg bw	– No mortalities. – Moderate to severe erythema and barely perceptible edema were noted in all treated animals. Edema cleared by Day 13, but erythema persisted in some animals at study termination. – Slight exfoliation and slight to moderate fissuring was also observed on the application sites of all males and 4/5 females. – 2/5 female rabbits lost weight. – At necropsy, an enlarged spleen was observed in one male. – Microbial recovery assays were inconclusive due to problems with microbial contamination and difficulties interpreting data from animals that were orally treated with an antibiotic during acclimation. Microbial recovery data are not required for acute dermal studies. LOW TOXICITY ACCEPTABLE
Dermal irritation	Rabbit—New Zealand white – 1 male and 5 females treated with 500 mg TGAI or 2.8×10^{10} CFU (measured) in 0.5 mL saline, 2 sites (abraded and intact), 24-hour exposure – 2 sites remained untreated (abraded and intact)	**Intact** MIS 3.08/8 (48 h postapplication) MAS 2.58/8 (48, 72, 96 h postapplication) **Abraded** MIS 2.75/8 (48 h postapplication) MAS 2.67/8 (48, 72, 96 h postapplication)	– Very slight to moderate erythema and barely perceptible edema observed within 24 hours of application of the test material, i.e., at patch removal. – Erythema cleared on both intact and abraded sites by 14 days postapplication. – Edema cleared by 9 days postapplication on abraded sites and by 6 days postapplication on intact sites. – Fissuring was noted 72–96 hours after application on the abraded sites of two rabbits and on the intact sites of another rabbit. – One or both abraded sites of three rabbits and both intact sites of two rabbits displayed a shiny denuded area on Day 13. SUPPLEMENTAL due to the extended exposure duration, 24 hours.

STUDY	SPECIES, STRAIN AND DOSES	LD_{50}, NOAEL AND LOAEL	TARGET ORGAN, SIGNIFICANT EFFECTS, COMMENTS
ACUTE STUDIES			
Dermal irritation	Rabbit—New Zealand white – 3/sex treated with 500 mg TGAI in 0.6 mL saline (nominal: 1.8×10^{12} CFU), 2.5 × 2.5 cm, 4 hours	MIS 0.67/8 (1 h after patch removal) MAS 0.5/8 (24, 48, 72 h after patch removal)	– Very slight to well-defined erythema was observed at 1 hour and at 24 hours after patch removal. – Very slight edema was noted in one animal at 1 hour after patch removal. – No dermal irritation was noted at 48 hours after patch removal. SLIGHTLY IRRITATING ACCEPTABLE
Dermal sensitization	Guinea pig—albino – 10 animals intradermally injected with 0.025% TGAI (measured: 1.3×10^{10} CFU/g, TS), 3 injections/week, 10 inductions total – 10 animals intradermally injected with 0.025% heat-killed TGAI (KTS), 3 injections/week, 10 inductions total – 4 animals intradermally injected with 0.1% 1-chloro-2,4-dinitrobenzene (positive), 3 injections/week, 10 inductions total	POTENTIAL SENSITIZER	– Very slight to well-defined erythema and very slight edema was observed in all three groups after induction and challenge injections. – Dermal irritation scores were, on average, slightly greater than those observed during induction, but these results were equivocal. – Since all micro-organisms contain substances that could elicit a positive response in test animals, all MPCAs are considered as potential sensitization agents. – Dermal sensitization studies are not a guideline requirement for MPCAs. No replacement study is required. SUPPLEMENTAL

STUDY	SPECIES, STRAIN AND DOSES	LD_{50}, NOAEL AND LOAEL	TARGET ORGAN, SIGNIFICANT EFFECTS, COMMENTS
ACUTE STUDIES			
Eye irritation	Rabbit—New Zealand white – 6 rabbits treated with TGAI (measured: 5.5 × 10^9 CFU/g), 100 mg, right eye, unwashed – 3 rabbits treated with TGAI (measured: 5.5 × 10^9 CFU/g), 100 mg, right eye, washed with 20 mL tap water	**Unwashed** MIS 11.2/110 (24 h after instillation) MAS 7.2/110 (24, 48, 72 h after instillation) **Washed** MIS 2.7/110 (24 h after instillation) MAS 2.2/110 (24, 48, 72 h after instillation)	**Unwashed** – Conjunctival redness, chemosis and discharge were noted in the majority of animals. – Injected iris noted in one rabbit. – Irritation cleared by Day 10. – Purulent discharge was noted in 4/6 rabbits after 24 hours and in 2/6 rabbits after 48 hours. **Washed** – Conjunctival redness was noted in all 3 rabbits. – Irritation cleared by Day 10. MILDLY IRRITATING ACCEPTABLE
VectoLex WDG			
Oral toxicity	Rat—Sprague Dawley, young adult – 5/sex treated with VectoLex WDG in deionized water at 5050 mg/kg bw, gavage	LD_{50} greater than 5050 mg/kg bw	– No mortalities – Decreased defecation noted in 2/10 animals after 2 hours. – One male rat lost weight between Days 7 and 14. – At necropsy, mottled lungs were noted in 3/5 males and 5/5 females, and mottled kidneys were noted in one female and one male. – Study author noted that these findings may be related to an infectious process known to occur, without clinical signs, in rats of this age. – Lung discolourations may also be a normal immunological reaction to a foreign antigen (due to dosing error or accidental aspiration of the gavage material) or a toxicological reaction to the formulation ingredients present in VectoLex WDG. – No kidney effects were noted in any of the other studies with *Bacillus sphaericus* strain 2362. LOW TOXICITY ACCEPTABLE

STUDY	SPECIES, STRAIN AND DOSES	LD_{50}, NOAEL AND LOAEL	TARGET ORGAN, SIGNIFICANT EFFECTS, COMMENTS
ACUTE STUDIES			
Inhalation toxicity	Rat—Sprague Dawley – 5/sex treated with VectoLex WDG, 4 hours, nose-only – 0.435 mg/L, MMAD = 0.6 μm, GSD = 110.5	LC_{50} greater than 0.435 mg/L	– No mortalities – No signs of toxicity – No lesions at necropsy LOW TOXICITY ACCEPTABLE
Dermal toxicity	Rabbit—New Zealand white – 5/sex treated with 5050 mg/kg bw VectoLex WDG in 1.08 mL/g deionized water to at least 10% of the body surface, 24 hours, washed	LD_{50} greater than 5050 mg/kg bw	– No mortalities – One male rabbit exhibited decreased defecation 4 hours after dosing. – Females exhibited soft feces 1–4 hours after dosing. – Moderate to severe erythema and slight to moderate edema was observed on all animals at 1 hour after patch removal. – Edema cleared by Day 4 and erythema cleared by Day 14. – Focal areas of bleeding, bruising, necrosis, desquamation, coriaceousness, eschar formation and shallow lateral fissuring were observed on Days 1, 4, 7 and 11. – One female failed to gain weight. – No lesions at necropsy LOW TOXICITY ACCEPTABLE
Dermal irritation	Rabbit—New Zealand white – 3/sex treated with 500 mg VectoLex WDG in 0.5 mL deionized water, 2.5 × 2.5 cm, 4 hours	MIS 0.83/8 (1 h after patch removal) MAS 0.11/8 (24, 48, 72 h after patch removal)	– Very slight erythema was observed in 2/3 males and 2/3 females at 1 hour, and in 1 male and in 1 female at 24 hours after patch removal. – Very slight edema was noted in 1 animal at 1 hour after patch removal. SLIGHTLY IRRITATING ACCEPTABLE
Eye irritation	Rabbit—New Zealand white – 3/sex instilled with 0.1 mL VectoLex WDG (44.6 mg), right eye, unwashed	MIS 5.3/110 (1 h after instillation) MAS 1.1/110 (24, 48, 72 h after instillation)	– Conjunctival irritation was observed in the eyes of all rabbits at the 1-hour observation. MINIMALLY IRRITATING ACCEPTABLE

Appendix II Environmental Assessment

Table 1 Summary of Effects on Terrestrial Organisms

ORGANISM	EXPOSURE	TEST SUBSTANCE	ENDPOINT VALUE	SIGNIFICANT EFFECTS, COMMENTS
Vertebrates				
Birds	Oral	*Bacillus sphaericus* serotype H-5a5b (*Bacillus sphaericus* strain 2362)	LD_{50} > 9g/kg bw or > 2.98×10^{13} CFU/kg bw	– No mortalities – No signs of toxicity – No findings at necropsy LOW TOXICITY SUPPLEMENTAL because pathogenicity not addressed – Lack of clinical signs at dose 4.5 × limit dose suggests the MPCA not pathogenic
	Dietary	ABG-6184 Technical Powder; active ingredient *Bacillus sphaericus* (*Bacillus sphaericus* strain 2362)	30-day dietary LD_{50} > 2.4×10^{6} CFU/kg bw/day (approximate dose calculated from theoretical consumption of diet containing 20% TS by weight)	– No mortalities – No signs of toxicity – No findings at necropsy LOW TOXICITY SUPPLEMENTAL because pathogenicity not addressed – Lack of clinical signs despite high dietary dose for 30 consecutive days suggests the MPCA not pathogenic
	Intraperitoneal Injection	*Bacillus sphaericus* serotype H-5a5b (*Bacillus sphaericus* strain 2362)	N/A	UNACCEPTABLE because of poor study design – Not considered in the risk assessment.
Wild mammals	Data requirement waived based on the results of human health and safety testing data.			

ORGANISM	EXPOSURE	TEST SUBSTANCE	ENDPOINT VALUE	SIGNIFICANT EFFECTS, COMMENTS
Invertebrates				
Bees	Dietary	Spherimos Technical (*Bacillus sphaericus* strain 2362)	Dietary study LD_{50} > 5×10^7 CFU/mL	– Terminated on Day 7 due to >20% mortality in controls – Rate of mortality comparable in untreated control and treated replicates LOW TOXICITY ACCEPTABLE
	Dietary	ABG-6184 (*Bacillus sphaericus* strain 2362)	LC_{50} not calculated (see effects)	– Mortality in control groups exceeded 20% by Day 4 (Experiment 1) or Day 10 (Experiment 2) – Rate of mortality and time to death similar in control and treated groups LOW TOXICITY ACCEPTABLE
Ladybird beetles	Dietary	Spherimos Technical (*Bacillus sphaericus* strain 2362)	Dietary study LD_{50} for solution into which pollen grains were dipped > 1.5 mg/L	– No mortalities – No signs of toxicity SUPPLEMENTAL for the following reasons: – No confirmation of MPCA viability – Quantity of dosing solution on dipped pollen grains not determined – Very low dose used
Crickets	Dietary	Spherimos Technical (*Bacillus sphaericus* strain 2362)	Dietary study LD_{50} for solution into which apple slices were dipped > 1.5 mg/L	– No mortalities – No signs of toxicity SUPPLEMENTAL for the following reasons: – No confirmation of MPCA viability – Quantity of dosing solution on dipped apple slices not determined – Very low dose used

ORGANISM	EXPOSURE	TEST SUBSTANCE	ENDPOINT VALUE	SIGNIFICANT EFFECTS, COMMENTS
Bioassays	Dietary	*Bacillus sphaericus* TGAI (*Bacillus sphaericus* strain 2362)	Dietary bioassays: LD_{50} not determined (see significant effects, comments)	– *Trichoplusia ni* 96.7% mortality dose of 3 g/L in diet; 13.7% mortality at dose of 300 mg/L in diet – *Leptinotarsa decemlineata* 11.4% corrected mortality when fed leaves dipped in 10 g/L suspension SUPPLEMENTAL – A replacement bioassay is required
Earthworms and other non-arthropod invertebrates	Data waiver granted based on the host range and mode of action of the MPCA, on a literature survey showing no reports of adverse effects in non-target, non-arthropod invertebrates, except for the inhibition of hatching of nematode eggs by a spore extract from a closely-related strain.			
Soil microbes	Data were not required as VectoLex products are not intended to control pest micro-organisms. The biology, ecology and proposed use pattern indicate little potential for adverse effects on environmentally or economically important microbial species or microbiologically mediated biogeochemical processes.			
Vascular Plants				
Vascular plants	A request to waive plant testing requirements was granted based on the host range and mode of action of the MPCA.			

Table 2 Summary of Effects on Aquatic Organisms

ORGANISM	EXPOSURE	TEST SUBSTANCE	ENDPOINT VALUE	SIGNIFICANT EFFECTS, COMMENTS
Vertebrates				
Rainbow trout	Acute toxicity	"Technical Grade of *Bacillus sphaericus* H-5a5b" (*Bacillus sphaericus* strain 2362)	96-hour LC_{50}/EC_{50} > 15.5 mg/L (nominal concentration of 6.2×10^5 CFU/L)	– No mortalities – No signs of toxicity SUPPLEMENTAL because neither the MCC for microbial hazard testing nor the limit dose for chemical toxicity testing were used.
Bluegill sunfish	Acute toxicity	"Technical Grade of *Bacillus sphaericus* H-5a5b" (*Bacillus sphaericus* strain 2362)	96-hour LC_{50}/EC_{50} > 15.5 mg/L (nominal concentration of 6.2×10^5 CFU/L)	– No mortalities – No signs of toxicity SUPPLEMENTAL because neither the MCC for microbial hazard testing nor the limit dose for chemical toxicity testing were used.
Bluegill sunfish	Pathogenicity/ infectivity	"Unstabilized spray-dried fermentation product of *Bacillus sphaericus*" (*Bacillus sphaericus* strain 2362)	32-day LC_{50} > 4.41×10^{10} CFU/L (aquatic)/4.41×10^9 CFU/g (dietary).	– One mortality (1/30) in the treated group – Survival was not significantly different between treated and control groups – Mean growth rate significantly lower in test groups, probably due to turbidity in the test solution – Necropsies and histopathological observations were normal ACCEPTABLE
Sheepshead minnow	Acute toxicity	"*Bacillus sphaericus* (ABG-6184) Technical Material" (*Bacillus sphaericus* strain 2362)	96-hour LC_{50} > 100 mg/L (nominal 7.9×10^9 CFU/L) NOEC 22 mg/L.	– No mortality – Increased surfacing in the groups exposed to the TS at 36, 60 and 100 mg/L, probably due to a rapid, dose-dependent decrease in dissolved oxygen observed between renewals SUPPLEMENTAL because pathogenicity was not addressed.

ORGANISM	EXPOSURE	TEST SUBSTANCE	ENDPOINT VALUE	SIGNIFICANT EFFECTS, COMMENTS
Sheepshead minnow	Pathogenicity/ infectivity	"Unstabilized spray-dried fermentation product of *Bacillus sphaericus*" (*Bacillus sphaericus* strain 2362)	32-day LC_{50} > 4.41×10^{10} CFU/L (aquatic)/4.41×10^{9} CFU/g (dietary)	– No mortality – No treatment signs of toxicity – Necropsy and histopathological analyses were normal ACCEPTABLE
Invertebrates				
Daphnid	96-h acute toxicity	"*Bacillus sphaericus* (ABG-6184) Technical Material" (*Bacillus sphaericus* strain 2362)	96-hour LC_{50} > 15.5 mg/L (nominal 6.2×10^{8} CFU/L)	– No mortality – No sublethal effects SUPPLEMENTAL because neither the MCC for microbial hazard testing nor the limit dose for chemical toxicity testing were used.
Mayfly	96-h acute toxicity	"*Bacillus sphaericus* (ABG-6184) Technical Material" (*Bacillus sphaericus* strain 2362)	96-hour LC_{50} > 15.5 mg/L (nominal 6.2×10^{8} CFU/L)	– No mortality – No sublethal effects SUPPLEMENTAL because neither the MCC for microbial hazard testing nor the limit dose for chemical toxicity testing were used.
Daphnid	21-d toxicity/ pathogenicity	"Unstabilized spray-dried fermentation product of *Bacillus sphaericus*" (*Bacillus sphaericus* strain 2362)	21-day NOEC 4.86×10^{9} CFU/L (measured)	– 21-day EC_{50} for immobility 2.82×10^{9} CFU/L (95% CI: 1.91×10^{10} to 4.93×10^{10} CFU/L) ACCEPTABLE
Chironomid	21-d toxicity/ pathogenicity	"*Bacillus sphaericus* TGAI" (*Bacillus sphaericus* strain 2362)	21-day EC_{50} > 7.6×10^{10} CFU/L	– Survival was not significantly different between test and untreated control groups. ACCEPTABLE
Mysid	96-h acute toxicity	*Bacillus sphaericus* strain 2362	96-hour NOEC 50 mg/L (nominal 4.0×10^{9} CFU/L)	– 96-hour LC_{50} > 71 mg/L (95% CI: 50 to 100 mg/L) ACCEPTABLE

ORGANISM	EXPOSURE	TEST SUBSTANCE	ENDPOINT VALUE	SIGNIFICANT EFFECTS, COMMENTS
Oyster	Acute toxicity/ shell deposition	"*Bacillus sphaericus* technical material" (*Bacillus sphaericus* strain 2362)	96-hour NOEC for shell deposition 15 mg/L (measured 8.6 × 10^8 CFU/L)	– 96-hour EC_{50} for shell deposition 42 mg/L (95% CI: 26 to 72 mg/L) ACCEPTABLE – The NOEC is equivalent to an EEC that can reasonably be expected following repeated application of the MPCA. – A long-term study in Eastern oyster or a susceptible Canadian freshwater bivalve mollusc is required.
Plants				
Unicellular alga	Acute	"*Bacillus sphaericus* technical material" (*Bacillus sphaericus* strain 2362)	120-hour EC_{50} > 2.2 mg/L (nominal 1.7 × 10^8 spores/L)	– No significant difference in growth between test and untreated control groups SUPPLEMENTAL because neither the MCC nor the toxicity testing limit dose were used.

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