

Public Release Summary

on

Evaluation of the new active

***Bacillus sphaericus* STRAIN 2362**

in the product

VECTOLEX WG BIOLOGICAL LARVICIDE

Australian Pesticides and Veterinary Medicines Authority

November 2004

**Canberra
Australia**

©Australian Pesticides and Veterinary Medicines Authority 2004
ISSN1443-1335

This work is copyright. Apart from any use permitted under the *Copyright Act 1968*, no part may be reproduced without permission from the Australian Pesticides and Veterinary Medicines Authority. Requests and enquiries concerning reproduction and rights should be addressed to the Manager, Communication and Secretariat, Australian Pesticides and Veterinary Medicines Authority, PO Box E240, Kingston ACT 2604 Australia.

This document is published by the Australian Pesticides and Veterinary Medicines Authority. In referencing, the APVMA should be cited as both the author and publisher of this document. For further information, please contact:

Jenny Dunn
Australian Pesticides and Veterinary Medicines Authority
PO Box E 240
KINGSTON ACT 2604

Ph: (02) 6272 5890
Fax: (02) 6272 3218

FOREWORD

The Australian Pesticides and Veterinary Medicines Authority (APVMA) is an independent statutory authority with responsibility for assessing and approving agricultural and veterinary chemical products prior to their sale and use in Australia.

In undertaking this task, the APVMA works in close cooperation with advisory agencies, including the Department of Health and Ageing (Office of Chemical Safety), Department of Environment and Heritage (Risk Assessment and Policy Section), the National Occupational Health and Safety Commission (Worksafe Australia) and State departments of agriculture and environment.

The APVMA has a policy of encouraging openness and transparency in its activities and of seeking community involvement in decision making. Part of that process is the publication of public release summaries for all products containing new active ingredients and for all proposed extensions of use for existing products.

The information and technical data required by the APVMA to assess the safety of new chemical products and the methods of assessment must be undertaken according to accepted scientific principles. Details are outlined in the APVMA's publications *Ag Manual: The Requirements Manual for Agricultural Chemicals* and *Ag Requirements Series*.

This Public Release Summary is intended as a brief overview of the assessment that has been completed by the APVMA and its advisory agencies. It has been deliberately presented in a manner that is likely to be informative to the widest possible audience thereby encouraging public comment.

More detailed technical assessment reports on all aspects of the evaluation of this chemical can be obtained by completing the order form in the back of this publication and submitting with payment to the APVMA. Alternatively, the reports can be viewed at the APVMA Library, 1st Floor, 22 Brisbane Avenue, Barton, ACT.

The APVMA welcomes comment on the usefulness of this publication and suggestions for further improvement. Comments should be submitted to the Program Manager—Pesticides Division, Australian Pesticides and Veterinary Medicines Authority, PO Box E240, Kingston ACT 2604.

[blank page here]

CONTENTS

Foreword	iii
List of Abbreviations and Acronyms	7
Introduction	9
Chemistry and Manufacture	11
Active Constituent	11
Formulated Product	11
Physical and Chemical Properties of the Product	12
Summary of the Chemistry Evaluation of VectoLex WG Biological Larvicide	12
Toxicological Assessment	13
Evaluation of Toxicity	13
Acute Studies	13
Other Studies	13
Poisons Scheduling	14
ADI/ARfD	14
Residues Assessment	15
Assessment of Overseas Trade Aspects of Residues in Food	15
Occupational Health and Safety Assessment	17
Assessment of Occupational Health and Safety	17
Formulation, packaging, transport, storage and retailing	17
Use and Exposure	17
Entry into treated areas	18
Recommendations for safe use	18
Re-entry Statement	18
Conclusion	18
Environmental Assessment	19
Natural Occurrence in Australia	19
Toxins	19
Mode of Action	19
Host Range	20
Development of Resistance	20
Persistence and Activity in the Environment	20
Potential for Horizontal Gene Transfer	21
Conclusion	21
Efficacy and Safety Assessment	22
Justification and Proposed Use Pattern	22
Evaluation of Efficacy	22

Phytotoxicity	22
Conclusion	22
Labelling Requirements	23
Glossary	27
References	28
APVMA Publications Order Form	29

LIST OF ABBREVIATIONS AND ACRONYMS

AC	active constituent
ACR	Acute to chronic ratio
ACTH	Adrenocorticotrophic hormone
ADI	Acceptable Daily Intake (for humans)
AHMAC	Australian Health Ministers Advisory Council
ai	active ingredient
ALT	Alanine aminotransferase
ARfD	Acute Reference Dose (for humans)
BBA	Biologische Bundesanalstalt fur Land – und forstwirtschaft
bw	bodyweight
CHO	Chinese hamster ovary
CRP	Chemistry and Residues Program
d	day
DAT	Days After Treatment
DM	Dry Matter
DT₅₀	Time taken for 50% of the concentration to dissipate
DT₉₀	Time taken for 90% of the concentration to dissipate
EA	Environment Australia
E_bC₅₀	concentration at which the biomass of 50% of the test population is impacted
EC₅₀	concentration at which 50% of the test population are immobilised
EEC	Estimated Environmental Concentration
E_rC₅₀	concentration at which the rate of growth of 50% of the test population is impacted
EUP	End Use Product
F₀	original parent generation
FW	Fresh Weight
g	gram
GAP	Good Agricultural Practice
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GVP	Good Veterinary Practice
h	hour
ha	hectare
Hct	Heamatocrit
HDPE	High-density polyethylene
Hg	Haemoglobin
HPLC	High Pressure Liquid Chromatography <i>or</i> High Performance Liquid Chromatography
HPLC-UV	High Performance Liquid Chromatography with Ultra-Violet Detector
id	intradermal
im	intramuscular
ip	intraperitoneal
IPM	Integrated Pest Management
iv	intravenous
in vitro	outside the living body and in an artificial environment

in vivo	inside the living body of a plant or animal
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
Kg	kilogram
K_{oc}	Organic carbon partitioning coefficient
L	Litre
LC₅₀	concentration that kills 50% of the test population of organisms
LD₅₀	dosage of chemical that kills 50% of the test population of organisms
LC-MS/MS	liquid chromatography, mass spectroscopy
LOEC	Lowest Observable Effect Concentration
LOD	Limit of Detection – level at which residues can be detected
LOQ	Limit of Quantitation – level at which residues can be quantified
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
mg	milligram
mL	millilitre
MOE	Margin of Exposure
MRL	Maximum Residue Limit
MSDS	Material Safety Data Sheet
NDPSC	National Drugs and Poisons Schedule Committee
NEDI	National Estimated Daily Intake
NESTI	National Estimated Short Term Intake
Ng	nanogram
NHMRC	National Health and Medical Research Council
NOEC/NOEL	No Observable Effect Concentration Level
OC	Organic Carbon
OM	Organic Matter
PHED	Pesticide Handlers Exposure Database
PHI	Pre-harvest interval
po	oral
POEM	Predictive Operator Exposure Model (UK)
ppb	parts per billion
PPE	Personal Protective Equipment
ppm	parts per million
Q-value	Quotient-value
RBC	Red Blood Cell Count
S	second
sc	subcutaneous
SC	Suspension Concentrate
STMTR	Supervised Trial median Residue
SUSDP	Standard for the Uniform Scheduling of Drugs and Poisons
TGA	Therapeutic Goods Administration
TRR	Total Radioactive residues
T-Value	A value used to determine the First Aid Instructions for chemical products that contain two or more poisons
µg	microgram
WG	Water Dispersible Granule
WHO	World Health Organisation
WHP	Withholding Period

INTRODUCTION

This publication provides a summary of the data reviewed and an outline of the regulatory considerations for the proposed registration of *VECTOLEX WG BIOLOGICAL LARVICIDE*, which contains the new active constituent *Bacillus sphaericus* strain 2362. The product is proposed to be used for the control of certain first to early fourth instar mosquito larvae in a range of mosquito breeding situations.

Responses to this Public Release Summary will be considered prior to registration of the product. They will be taken into account by the APVMA in deciding whether the product should be registered and in determining appropriate conditions of registration and product labelling.

Copies of full technical evaluation reports for *Bacillus sphaericus* strain 2362 covering toxicology, occupational health and safety aspects, and environmental aspects are available from the APVMA on request (see order form on last page). They can also be viewed at the APVMA library located at the APVMA offices, First Floor, 22 Brisbane Avenue, Barton ACT 2604.

Written comments should be received by the APVMA by 7 December 2004. They should be addressed to:

Jenny Dunn
Pesticides Division
Australian Pesticides and Veterinary Medicines Authority
PO Box E240
KINGSTON ACT 2604

Phone: (02) 6272 5890
Fax: (02) 6272 3218
Email: jennifer.dunn@apvma.gov.au

Applicant

Valent BioSciences, a division of Sumitomo Chemical Australia Pty Ltd.

Product Details

It is proposed to register *VECTOLEX WG BIOLOGICAL LARVICIDE*, containing *Bacillus sphaericus* strain 2362 as a water dispersible granule formulation. *VECTOLEX WG BIOLOGICAL LARVICIDE* will be imported fully formulated and packaged in 500 g, 10 kg and 25 kg containers.

Bacillus sphaericus strain 2362 is a naturally occurring, spore-forming bacterium found in soil and aquatic environments. At the time of sporulation, it produces delta-endotoxin, which on ingestion causes disruption of the midgut epithelium leading to death of susceptible larvae of certain mosquito species. With respect to insecticide resistance, *Bacillus sphaericus* strain 2362 is classed as a Group 11D insecticide.

Application is via ground or aerial application of spray for the control of *Culex* spp. and *Anopheles* spp. in a range of aquatic mosquito breeding habitats, such as stagnant and standing ponds, flood and irrigation water, ditches, storm water retention areas, tidal water and salt marshes, sewerage settling ponds and water with moderate to high organic content.

Products containing *Bacillus sphaericus* strain 2362 are currently registered in Argentina, Brazil, Bulgaria, Columbia, Dominican Republic, El Salvador, Mexico, Peru, Romania, Singapore, Thailand, Turkey, USA and Venezuela.

CHEMISTRY AND MANUFACTURE

Active constituent

Bacillus sphaericus strain 2362 is a naturally occurring spore-forming insecticidal bacterium. During sporulation, *Bacillus sphaericus* produces delta-endotoxin, which binds specifically and with high affinity to the midgut epithelial receptors of mosquito larvae, leading to death of susceptible larvae.

The active constituent *Bacillus sphaericus* strain 2362 is generated by propagation from a seed culture and formulated into the product VectoLex WG Biological Larvicide in an integrated process, i.e. the active constituent is not usually separately isolated. The dried concentrate of *Bacillus sphaericus* strain 2362 has the following properties:

Common name:	<i>Bacillus sphaericus</i> strain 2362
IUPAC name:	Not applicable
CAS Registry Number:	Not applicable
Family:	Bacillaceae
Genus:	<i>Bacillus</i>
Species:	<i>sphaericus</i>
Serotype:	H5a5b
Strain:	2362
Appearance:	Brown powder with a characteristic musty odour
Melting point:	Not applicable
Density:	0.52 g/cm ³
pH:	6.3 (10% aqueous slurry)
Vapour pressure:	Negligible
Active type:	Insecticide
Mode of action:	Secretion of delta-endotoxin, which binds to the midgut epithelial receptors of mosquito larvae, leading to the death of susceptible larvae.

The Chemistry and Residues Program (CRP) of the APVMA has evaluated the biological and manufacturing aspects of *Bacillus sphaericus* strain 2362 (manufacturing process, quality control procedures, batch analysis results and analytical methods) and found them to be acceptable.

Bacillus sphaericus strain 2362 is a new active constituent and there is no compendial specification available. An Active Constituent Standard has not been established for *Bacillus sphaericus* strain 2362.

Formulated product

The CRP of the APVMA has assessed the chemistry and manufacturing data submitted for the formulated product.

Distinguishing name:	VectoLex WG Biological Larvicide
Formulation type:	Water-dispersible granule

Physical and chemical properties of the product

Appearance:	Brown free-flowing granules
Bulk density:	0.3-0.5 g/cm ³
pH:	4.0-6.0 (10% slurry)
Safety properties:	Not corrosive and not classified as a dangerous good
Storage stability:	Stable for at least 24 months when stored below 25 °C (air conditioning)

Summary of the chemistry evaluation of VectoLex WG Biological Larvicide

VectoLex WG Biological Larvicide will be formulated in the United States.

The manufacturing and quality control procedures, including compliance with the release specifications, are acceptable.

Agricultural chemical products containing organisms (including *Bacillus sphaericus*) are date-controlled. The applicant provided the results of real time and accelerated stability testing conducted using samples stored in HDPE bottles. Testing of all of the important parameters for water-dispersible granule formulations was conducted. The results indicate that the formulated product is expected to be stable for at least 24 months when stored below 25 °C (air conditioning) in the proposed commercial packaging. Therefore, the APVMA has assigned a shelf life of 24 months, with storage below 25 °C, to VectoLex WG Biological Larvicide.

Based on a review of the data provided by the applicant to the APVMA, the APVMA is satisfied that the chemistry and manufacturing details of VectoLex WG Biological Larvicide are acceptable.

TOXICOLOGICAL ASSESSMENT

Evaluation of toxicology

The toxicological database for *Bacillus sphaericus*, which consists primarily of toxicity tests conducted using animals, is reasonable. In interpreting the data, it should be noted that toxicity tests generally use doses that are high compared with likely human exposures. The use of high doses increases the likelihood that potentially significant toxic effects will be identified. Findings of adverse effects in any one species do not necessarily indicate such effects might be generated in humans. From a conservative risk assessment perspective however, adverse findings in animal species are assumed to represent potential effects in humans, unless convincing evidence of species specificity is available. Where possible, considerations of the species-specific mechanisms of adverse reactions weigh heavily in the extrapolation of animal data to likely human hazard. Equally, consideration of the risks to human health must take into account the likely human exposure levels compared with those, usually many times higher, which produce effects in animal studies. Toxicity tests should also indicate dose levels at which the specific toxic effects are unlikely to occur. Such dose levels as the No-Observable-Effect-Level (NOEL) are used to develop acceptable limits for dietary or other intakes, Acceptable Daily Intake (ADI) and Acute Reference Dose (ARfD), at which no adverse health effects in humans would be expected.

Acute Studies

Bacillus sphaericus has low acute oral ($LD_{50} > 5000$ mg/kg bw in rats) and dermal toxicity ($LD_{50} > 2000$ mg/kg bw in rabbits). In an acute inhalation toxicity study in rats, the LC_{50} was greater than 90 mg/M³ with no deaths (the maximum achievable concentration). *Bacillus sphaericus* is a slight eye irritant and a slight skin sensitiser but not a skin irritant.

Viable organisms were found in the urine, faeces and tissues of treated rats up to 7 days after a single oral dose of *Bacillus sphaericus*, but they were absent by 14 days post-dose. 24 hours after intravenous injection of *Bacillus sphaericus*, the organisms were detected in the liver, lungs, spleen, kidney, blood, lymph nodes and brain. The lungs, liver and spleen had the greatest concentrations of the organisms. The organisms were however completely cleared from the tissues by day 21 after treatment, except for the liver, spleen and lungs in which the organisms were eliminated by day 49. Rats receiving a single dose of *Bacillus sphaericus* by intra-tracheal instillation showed the organisms predominantly in the lungs, but they were eliminated by day 49 after treatment. At necropsy, discolouration of the lungs and treatment-related respiratory tract lesions (red/grey spots on the lung or mottled lungs) were observed in some treated rats.

The product VectoLex WG Biological Larvicide has low acute oral and dermal toxicity in rats and rabbits (LD_{50} is greater than 5050 mg/kg bw). The acute inhalation toxicity study showed that VectoLex WG Biological Larvicide had a LC_{50} of greater than 435 mg/M³ in rats, which is the maximum achievable concentration. In addition, VectoLex WG Biological Larvicide was a slight eye irritant but was not a skin irritant.

Other Studies

The administration of 10^7 to 10^8 spores of *Bacillus sphaericus* (strains 1593 or 2297) to mice or rats by conventional routes did not cause any changes in mortality, clinical signs, body weight gain and gross pathology parameters. Increases in spleen or liver weights were detected in some animals given *Bacillus sphaericus* strains 1593 or 2297 intravenously or

intraperitoneally. Similarly, there were no mortality, clinical illness and gross lesions in animals given 3×10^8 - 7×10^9 CFU *Bacillus sphaericus* (strains 1593, SSII and 1404) by subcutaneous or intraperitoneal injection. In a study involving topical ocular instillation of *Bacillus sphaericus* in rabbit conjunctivae, the organisms continued to be recovered 8 weeks after treatment, but there was no evidence for infection. Taken together, these studies show that *Bacillus sphaericus* strains 2297, SSII, 1404 and 1593 at high doses have no adverse effects when given by conventional routes to animals.

Studies on human isolates further demonstrated that *Bacillus sphaericus* was not a pathogen. Mice were given 9×10^6 - 9×10^8 *Bacillus sphaericus* (collected from an abscess of a human lung) by subcutaneous injection. 10-14 days after treatment, the animals were killed and examined grossly. No animals were ill or died as a result of injection with this material. Furthermore, there were no lesions seen at necropsy, except for a parvovarian cyst seen on the ovary of one animal. No *Bacillus sphaericus* was recovered from any of the treated animals. Similar results were obtained when mice were treated by intraperitoneal injection with *Bacillus sphaericus* derived from the human lung. These studies suggested that *Bacillus sphaericus* recovered from the human lung are not primary pathogens. The World Health Organisation have reported that there is no evidence that *Bacillus sphaericus* is a human or animal pathogen.

Poisons Scheduling

The National Drugs and Poisons Schedule Committee (NDPSC) considered the toxicity of the product and the active ingredient. The NDPSC decided that *Bacillus sphaericus* should be exempt from poisons scheduling. There are provisions for appropriate warning statements on the product label.

ADI/ARfD

An ADI or ARfD cannot be established due to insufficient data. However, *Bacillus sphaericus* strain 2362 is not intended to be used in food production.

RESIDUES ASSESSMENT AND ASSESSMENT OF OVERSEAS TRADE ASPECTS OF RESIDUES IN FOOD

Introduction

The Chemistry and Residues Program of the APVMA has undertaken a residue assessment of the proposed registration of VectoLex WG Biological Larvicide, containing *Bacillus sphaericus* strain 2362. The product is for specific control of certain first to early fourth instar mosquito larvae in ponds, water retention areas, tidal water and salt marshes at treatment rates of 0.5-1.5 kg/ha. Toxicology and environmental fate were considered as part of the residue evaluation of *Bacillus sphaericus* strain 2362.

Metabolism

The metabolism of *Bacillus sphaericus* strain 2362 is not required to be assessed further due to the treatment of water having no direct effect on non-target plants and animals. Furthermore, toxicology assessment indicates *Bacillus sphaericus* is not a human or animal pathogen.

Residue definition

As the active constituent is a biological control agent, a residue definition for *Bacillus sphaericus* is not required for this application.

Residue trials

As a biological control agent for the treatment of water, there is no likelihood of detectable residues being present in food and animal feeds from indirect exposure to the proposed use.

Bacillus sphaericus strain 2362 complies with the APVMA guidelines for the registration of biological agricultural products and is similar to other biological larvicides, ie VectoBac WG Biological Larvicide, P52642. As there are no toxicological concerns regarding registration, a Table 5 entry is supported for *Bacillus sphaericus* strain 2362.

Animal commodity and animal feed MRLs

The toxicology report indicates *Bacillus sphaericus* has a low acute toxicology profile and showed no potential for infectivity. Following oral exposure, viable organisms were found in the urine, faeces, and many tissues of treated rats. It is noted that elimination of the organisms from the tissues was slow, however, multiplication of the organism was not observed, suggesting lack of pathogenicity.

Estimated dietary intake

The proposed use is for treatment of non-potable water, and accordingly, human dietary exposure should not occur following the use of VectoLex WG Biological Larvicide as per the label directions.

Recommendations

The following amendments to the *MRL Standard* are recommended in relation to the proposed use of VectoLex WG Biological Larvicide:

Table 5

Compound	Use
ADD: <i>Bacillus sphaericus</i> strain 2362	Mosquito control in water

A withholding period is not required for VectoLex WG Biological Larvicide.

Assessment of overseas trade aspects of residues in food

Overseas registration status

Similar products containing *Bacillus sphaericus* strain 2362 have been registered overseas for mosquito larvae control.

Potential risk to Australian export trade

There are no trade implications arising from the registration of VectoLex WG Biological Larvicide, as treated waters (and products thereof) are not considered as major trade commodities.

OCCUPATIONAL HEALTH AND SAFETY ASSESSMENT

Assessment of Occupational Health and Safety

The National Occupational Health and Safety Commission (NOHSC) has conducted a risk assessment on VectoLex WG Biological Larvicide containing *Bacillus sphaericus* strain 2362 as water dispersible granules for the control of first to early fourth instar mosquito larvae belonging to *Culex* spp. and *Anopheles* spp. in a range of mosquito breeding situations. VectoLex WG Biological Larvicide can be safely used by workers when handled in accordance with the control measures indicated in this assessment.

Bacillus sphaericus has low acute oral and dermal toxicity in rats. It is a slight eye irritant in rabbits and a slight skin sensitiser in guinea pigs but was not a skin irritant in rabbits.

VectoLex WG Biological Larvicide will be applied to aquatic breeding habitats of mosquitoes by ground and aerial spraying. The maximum application rate is 1.5 kg/ha and suggested spray volumes are 2 L/ha for ULV application, 20 L/ha for thermal fogging and 50 – 150 L/ha for course spray. Reapplication at 14 – 21 day intervals is recommended.

Based on the risk assessment, cotton overalls buttoned to the neck and wrist and a washable hat, elbow-length PVC gloves and disposable dust mask should be worn when opening the container and preparing spray. Cotton overalls buttoned to the neck and wrist and a washable hat and elbow-length PVC gloves should be worn when applying the prepared spray by ground application.

Bacillus sphaericus is not on the NOHSC *List of Designated Hazardous Substances*. Based on the available information NOHSC has classified *Bacillus sphaericus* as hazardous substance according to the NOHSC *Approved Criteria for Classifying Hazardous Substances*. NOHSC has classified VectoLex WG Biological Larvicide as hazardous according to the NOHSC *Approved Criteria for Classifying Hazardous Substances*.

Bacillus sphaericus has low acute oral and dermal toxicity in rats. It is a slight eye irritant in rabbits and a slight skin sensitiser in guinea pigs but was not a skin irritant in rabbits.

VectoLex WG Biological Larvicide will be formulated overseas. It has low acute oral and dermal toxicity in rats. The product is a slight eye irritant in rabbits but not a skin irritant.

Formulation, packaging, transport, storage and retailing

The end-use product (EUP) will be imported into Australia fully packaged and ready for sale in 500g, 10kg and 25 kg containers.

Transport workers, store persons and retailers will handle the packaged product and could only become contaminated if the packaging were breached.

Use and exposure

VectoLex WG Biological Larvicide is indicated for the control of first to early fourth instar larvae of mosquitoes belonging to *Culex* spp. and *Anopheles* spp. in a range of aquatic breeding habitats of mosquitoes. The maximum application rate is 1.5 kg/ha. Suggested spray

volumes are 50-150 L/ha for course spray (hand sprayer, backpack mist blower), 20 L/ha for thermal flogging and 2 L/ha for ULV application. Reapplication at 14 – 21 day intervals is suggested.

The main routes of exposure are dermal, inhalation and ocular. Categories of workers that can be exposed to the product are mixer/loaders, ground applicators and clean-up personnel.

There are no available worker exposure data on VectoLex WG Biological Larvicide. The risk assessment indicates that the use of cotton overalls, gloves and disposable dust mask during mixing/loading and cotton overalls and gloves during ground application are necessary to protect workers from acute and repeated exposure.

Entry into treated areas

Workers are not expected to enter treated areas as treatment areas are stagnant and standing ponds, flood and irrigation water, ditches, storm water retention areas, tidal water and salt marshes, water with moderate to high organic content and sewerage settling ponds.

Recommendations for safe use

Users should follow the instructions and Safety Directions on the product label. Safety Directions include the use of cotton overalls buttoned to the neck and wrist, a washable hat, elbow-length PVC gloves and disposable mask when opening the container and preparing the spray and the use of cotton overalls buttoned to the neck and wrist, a washable hat and elbow-length PVC gloves when using the prepared spray for ground application.

The personal protective equipment recommended should meet the relevant Australian standards.

Re-entry statement

NOHSC do not recommend any re-entry statement.

Conclusion

NOHSC supports the registration of *Bacillus sphaericus* in VectoLex WG Biological Larvicide strain 2362 as a water dispersible granule formulation, for the control of first to early fourth instar mosquito larvae belonging to *Culex* spp. and *Anopheles* spp. in a range of mosquito breeding situations.

VectoLex WG Biological Larvicide can be used safely if handled in accordance with the instructions on the product label and any other control measures described above. Additional information is available in the VectoLex WG Biological Larvicide material safety data sheet.

ENVIRONMENTAL ASSESSMENT

VectoLex WG Biological Larvicide is a mosquito larvicide containing *Bacillus sphaericus*, a naturally occurring spore-forming bacterium that is found in soil and aquatic environments around the world. At the time of sporulation, *Bacillus sphaericus* produces delta-endotoxins that are toxic to many species of mosquito larvae upon ingestion. The mode of action of the *Bacillus sphaericus* delta-endotoxin is not fully characterized but involves the synergistic interaction of two peptides; one peptide binds to a receptor in the mosquito midgut epithelium while the other confers toxicity. *Bacillus sphaericus* persists in the environment longer than other commercially available larvicides. *Bacillus sphaericus* is reported to be among the most environmentally safe agents that are effective for controlling mosquito populations.

Natural occurrence in Australia

Due to the resistance of endospores to environmental stress and their long-term survival under adverse conditions, most aerobic spore formers are ubiquitous and can be isolated from a wide variety of sources. *Bacillus* spp. in soil become metabolically active when suitable substrates for growth are available and form spores when nutrients become exhausted. *Bacillus sphaericus* is a ubiquitous organism found throughout the world in soil and aquatic habitats. The strain used in the VectoLex WG Biological Larvicide formulation has not previously been registered for use in Australia.

Toxins

Many strains of *Bacillus sphaericus* produce toxins that are active against mosquito larvae. *Bacillus sphaericus* produces two classes of toxin, binary (Bin) toxins and mosquitocidal (Mtx) toxins. The Bin toxins are the most toxic of the *Bacillus sphaericus* toxins and determine the overall toxicity of different strains. High toxicity strains of *Bacillus sphaericus* always produce Bin toxins, and low toxicity strains produce only Mtx toxin or neither toxin. The *Bacillus sphaericus* strain in this product produces both the Bin toxin and Mtx toxin, and is among the most toxic of *Bacillus sphaericus* strains (Priest et al., 1997).

Mode of action

The mode of action of the Bin toxin of *Bacillus sphaericus* is not fully understood, however, the degree of toxicity is likely correlated with the binding affinity of the toxin to specific receptors in the midgut of the particular host. When ingested by mosquito larvae, one of the Bin peptides binds to a receptor with a unique binding site on the surface of epithelial cells, enabling the other Bin peptide to enter the larval gut where the toxin becomes active.

The Bin toxin of *Bacillus sphaericus* is considered a single-site acting molecule because of a single receptor interaction with the toxin. In order to prevent the development of resistance among mosquito populations, *Bacillus sphaericus* should not be used in isolation, but rather as part of an integrated pest management program. This would minimise continuous selection pressure from *Bacillus sphaericus* on mosquito populations and prevent the development of resistance (Charles and Nielsen-LeRoux, 2000; Regis et al., 2000).

Host range

Bacillus sphaericus is able to control mosquito populations and has little or no effect on non-target insects and other invertebrates inhabiting the same environment. *Bacillus sphaericus* is highly specific for all *Culex* spp. and most *Anopheles* spp.. It is not expected that birds, fish, microcrustaceans, and marine/freshwater/estuarine invertebrates that cohabit with the target mosquito larvae will be adversely affected from exposure to *Bacillus sphaericus* as a result of using VectoLex WG Biological Larvicide in the prescribed manner.

Development of resistance

Bacillus sphaericus has been used to control *Culex* spp. mosquito larvae in many areas of the world since the late 1980s. The organism has many advantages as a larvicide, including low environmental toxicity due to the high specificity of the toxins and high level of efficacy and persistence. Although the mode of action is not fully understood, the development of resistance in *Culex* spp. is thought to result from the toxin having a single receptor site in the mosquito midgut. It is generally accepted that continual use of a single insecticide favours the development of resistance in target populations.

The development of resistance to *Bacillus sphaericus* in mosquito populations is a growing concern. In order to prevent the development of resistance in *Culex* spp. populations, it is recommended that larvicides containing *Bacillus sphaericus* be used in an integrated pest management program (Zahiri et al., 2002; Mulla et al., 2003).

Persistence and activity in the environment

There are many properties that make *Bacillus sphaericus* suitable for use as a mosquito control agent. *Bacillus sphaericus* is reported to be among the most environmentally safe agents that are effective for controlling mosquito populations. *Bacillus sphaericus* has a narrow host-range, and does not adversely affect most non-target organisms. However, *Bacillus sphaericus* is persistent in the environment. The persistence is mainly due to its ability to colonise in the cadavers of host organisms. Other factors that affect the persistence of *Bacillus sphaericus* in the environment include UV light, agitation, water quality, pH and temperature.

Bacillus sphaericus is able to maintain its effectiveness for several weeks, however, the bacteria are sensitive to high temperature, high pH, UV light in the 300-400nm range, and high organic content. Since VectoLex WG Biological Larvicide is proposed for use in water and potentially in some soil environments, light does not play an important role in the inactivation of the Bin toxins. Dormant spores are resistant to extreme physical conditions and may exhibit longevity. *Bacillus sphaericus* may survive in the environment for up to 9 months without a decrease in toxicity.

Potential for horizontal gene transfer

Horizontal gene transfer between *Bacillus sphaericus* and other bacteria could facilitate the spread of genes for the Bin toxin in the environment. The Bin toxin genes are encoded by two highly conserved chromosomal genes (Baumann et al., 1991; Servant et al., 1999). Conjugal transfer of plasmid DNA is much more likely than homologous recombination of chromosomal DNA, therefore, the likelihood of homologous recombination occurring and presenting a significant risk to the environment is low.

Conclusion

VectoLex WG Biological Larvicide is very specific, targeting the mosquito larvae of *Culex* spp. and *Anopheles* spp.. The use of VectoLex WG Biological Larvicide in areas inhabited by mosquito larvae to control the spread of mosquito populations is unlikely to have an adverse impact on the survival of non-target organisms. Toxicological studies have demonstrated that *Bacillus sphaericus* is not likely to be harmful to a range of non-target organisms that cohabit with mosquito larvae, including birds, fish, microcrustaceans, marine/freshwater/estuarine invertebrates and insects. The Bin toxin genes are highly conserved chromosomal genes, and are unlikely to transfer to other microorganisms in the environment via homologous recombination. The mode of action of *Bacillus sphaericus* is not fully understood, however, it is known that the Bin toxin binds to a single receptor in the midgut epithelium of target species. Target species that have a single receptor interaction with the toxin are prone to develop resistance. The development of resistance in mosquito populations treated with *Bacillus sphaericus* has been observed in several countries. In order to prevent the development of resistance to *Bacillus sphaericus* in Australia, it is recommended that an appropriate integrated pest management program be implemented to minimise the risk of emergence of resistance to the Bin toxin.

EFFICACY AND SAFETY ASSESSMENT

Justification and Proposed Use Pattern

Bacillus sphaericus strain 2362 is a new larvicide belonging to the microbial disrupters of insect midgut membranes group. With respect to insecticide resistance, *Bacillus sphaericus* strain 2362 is classed as a Group 11D insecticide. VectoLex WG Biological Larvicide will provide users with an additional Group 11D insecticide for use in mosquito control programs.

The applicant proposes that VectoLex WG Biological Larvicide be used as a spray for the control of *Culex* spp. and most *Anopheles* spp. mosquito larvae in stagnant and standing ponds, flood and irrigation water, ditches, storm water retention areas, tidal water and salt marshes, sewerage settling ponds and water with moderate to high organic content. The proposed rate of use is 0.5 – 1.5 kg/ha applied in 2 to 150L water per hectare depending upon weather, spray equipment and mosquito habitat characteristics. The recommended minimum concentration of the product for application is 62.5 g/L.

VectoLex WG Biological Larvicide will be available in 500g, 10kg and 25kg containers.

Evaluation of Efficacy

The efficacy data presented support the claim of control of *Culex* spp. and *Anopheles* spp. in the aquatic situations specified above. The trial data were generally well presented and of high quality. Treatments were adequately replicated with appropriate controls. The trial data demonstrates that the rate of treatment of VectoLex WG Biological Larvicide that were presented were efficacious against *Culex* spp. and *Anopheles* spp..

Phytotoxicity

VectoLex WG Biological Larvicide is proposed for use in various aquatic situations. The label includes precautionary advice not to contaminate streams, rivers and waterways.

Conclusion

The data as presented were adequate to demonstrate the efficacy and crop safety aspects of the product when used for the control of *Culex* spp. and most *Anopheles* spp. mosquito larvae in stagnant and standing ponds, flood and irrigation water, ditches, storm water retention areas, tidal water and salt marshes, sewerage settling ponds and water with moderate to high organic content when used according to the proposed label instructions. Registration of this product is therefore recommended from a product efficacy standpoint.

LABELLING REQUIREMENTS

READ SAFETY DIRECTIONS BEFORE OPENING OR USING

VECTOLEX[®] WG BIOLOGICAL LARVICIDE


Active Constituent: *Bacillus sphaericus* Strain 2362

GROUP 11D INSECTICIDE

For specific control of certain first to early fourth instar mosquito larvae
as per the Directions for Use table

Net: 500g, 10kg, 25 kg



A division of  Sumitomo Chemical Australia Pty Ltd
ACN 081 096 255
501 Victoria Avenue (P.O. Box 5125)
Chatswood 2067 NSW Australia ph (02) 9904 6499

DIRECTIONS FOR USE

Restraint: DO NOT allow diluted spray to remain in the tank for more than 12 hours.

SITUATION	INSECT PEST	RATE OF TREATMENT	CRITICAL COMMENTS
Stagnant and standing ponds, flood and irrigation water, ditches, storm water retention areas, tidal water and salt marshes, water with moderate to high organic content, sewage settling ponds.	<i>Culex</i> spp. <i>Anopheles</i> spp.	0.5 - 1.5 kg/ha	<ol style="list-style-type: none"> 1. Apply in 2 to 150 L water per hectare in a minimum concentration of 62.5 g/L. 2. Use for control of 1st to early 4th instar. 3. Use lower rate when 1st to early 3rd instar larvae predominate. 4. Use higher rate when late 3rd to early 4th instar larvae predominate, or in areas of high organic matter such as sewage settling ponds. 5. Reapply at 14-21 day intervals but ensure that a resistance management strategy is in place (see additional information below).

NOT TO BE USED FOR ANY PURPOSE, OR IN ANY MANNER, CONTRARY TO THIS LABEL UNLESS AUTHORISED UNDER APPROPRIATE LEGISLATION.

GENERAL INSTRUCTIONS

Larvicidal activity, due to destruction of the midgut epithelium, occurs within 12 hours of ingestion. Because 4th instar larvae do not feed prior to pupation, application has to be made in the early stages of the life cycle. When more than 50% of the larval population is in the late 4th instar stage, the use of this product is not recommended.

INSECTICIDE RESISTANCE WARNING
GROUP 11D INSECTICIDE

For insecticide resistance management, VectoLex WG is a Group 11D insecticide.

Some naturally occurring insect biotypes resistant to VectoLex WG and other Group 11D insecticides may exist through normal genetic variability in any insect population. The resistant individuals can eventually dominate the insect population if VectoLex WG or other Group 11D insecticides are used repeatedly. The effectiveness of VectoLex WG on resistant individuals could be significantly reduced. Since occurrence of resistant individuals is difficult to detect prior to use, Valent BioSciences accepts no liability for any losses that may result from the failure of VectoLex WG to control resistant insects.

VectoLex WG may be subject to specific resistant management strategies. For further information contact your local supplier, Sumitomo Chemical Australia Pty Ltd representative or local agricultural department agronomist.

RESISTANCE MANAGEMENT

Repeated use of VectoLex WG (20 cycles) may lead to a level of resistance in some mosquito populations reducing the effectiveness of products containing *Bacillus sphaericus*. We recommend the use of an appropriate resistance management strategy which incorporates VectoBac (*Bacillus thuringiensis* var. *israelensis*) or other larvicides into the mosquito management programme.

Mixing

VectoLex WG may be applied in conventional ground or aerial application equipment with quantities of water sufficient to provide uniform coverage of the target area. The amount of water will depend on weather, spray equipment and mosquito habitat characteristics. Do not mix more VectoLex WG than can be used in a 12 hour period.

Fill the spray vat, mix tank etc with the desired quantity of water. Start agitation to provide moderate circulation before adding the required quantity of VectoLex WG into the vat. For extended operations check and clean the filters of mix tank regularly.

VectoLex WG suspends readily with water and will stay suspended over normal application periods. Recirculation may be necessary if the spray mixture has sat for several hours or longer.

AVOID CONTINUOUS AGITATION OF THE SPRAY MIXTURE DURING SPRAYING.

Do not mix with alkaline or dirty water.

For Ground Application,

For most ground spraying apply in 2 to 150 L water per hectare using hand pump, air blast, mist blower etc spray equipment.

For Aerial Application

VectoLex WG can be applied through fixed wing or helicopter aircraft equipped with either conventional boom or nozzle systems or rotary atomisers at a convenient dilution.

Rinse and flush spray equipment thoroughly following each use.

PROTECTION OF LIVESTOCK, WILDLIFE, FISH, CRUSTACEANS AND ENVIRONMENT:

Apart from labelled use according to the Directions for Use table, DO NOT contaminate streams, rivers or waterways with the chemical or used containers.

STORAGE AND DISPOSAL

Keep out of reach of children.

Store VectoLex WG in the closed, original container in a cool well-ventilated area. Do not store for prolonged periods in direct sunlight.

STORE BELOW 25^oC (AIR CONDITIONING).

This product is stable for 2 years if stored as indicated.

Triple rinse or preferably pressure rinse containers before disposal. Add rinsings to spray tank. Do not dispose of undiluted chemicals on site. If recycling, replace cap and return clean containers to recycler or designated collection point.

If not recycling, break, crush or puncture and bury empty containers in a local authority landfill. If no landfill is available, bury the containers below 500 mm in a disposal pit specifically marked and set up for this purpose clear of waterways, desirable vegetation and tree roots. Empty containers and product should not be burnt.

SAFETY DIRECTIONS

Will irritate the eyes. Repeated exposure may cause allergic disorders. Avoid contact with eyes, skin and open wounds. When opening the container and preparing spray, wear cotton overalls buttoned to the neck and wrist and a washable hat, elbow-length PVC gloves and disposable dust mask. When using the prepared spray, wear cotton overalls buttoned to the neck and wrist and a washable hat and elbow-length PVC gloves. Wash hands after use. After each day's use, wash gloves and contaminated clothing.

FIRST AID

If poisoning occurs contact a doctor or Poisons Information Centre. Phone Australia 13 11 26.

MATERIAL SAFETY DATA SHEET

Additional information is listed in the Material Safety Data Sheet.

EXCLUSION OF LIABILITY

Unless otherwise expressly stated in writing neither Valent BioSciences, Sumitomo Chemical Australia Pty Ltd ("the Companies") nor the distributor has any knowledge of the particular use to which the buyer proposes to put this product. In purchasing this product the buyer must rely solely upon his own skill and judgement as to its suitability for the particular purpose for which it is required. Except to the extent that exclusion or denial of liability is prohibited under the Trade Practices Act or any relevant state legislation, the Companies and the distributor expressly exclude any warranty as to the quality or fitness of any goods sold for any purpose whatsoever and deny all responsibility in contract tort negligence or otherwise for any harm or damage resulting from the use of such goods or from acting on the advice or recommendations as to such use given in good faith by any representative of the Companies or the distributor. If these conditions are unacceptable to the buyer, the goods should be returned to Sumitomo Chemical Australia Pty Ltd unopened within seven (7) days for refund of purchase price.

® Registered Trademark of Valent BioSciences Corporation, Libertyville, Illinois, USA

© Copyright of this label and every part of this label is owned by Valent BioSciences Corporation

THIS PRODUCT IS NOT CONSIDERED TO BE A DANGEROUS GOOD UNDER THE AUSTRALIAN CODE FOR THE TRANSPORT OF DANGEROUS GOODS BY ROAD OR RAIL.	
In a Transport Emergency Dial 000 Police or Fire Brigade	SPECIALIST ADVICE IN EMERGENCY ONLY ALL HOURS - AUSTRALIA WIDE 1800 024 973

AVPMA Approval No.: 55919/500g/

AVPMA Approval No.: 55919/10kg/

AVPMA Approval No.: 55919/25kg/

Batch No:

D.O.M.:

Expiry Date:

GLOSSARY

Active constituent	The substance that is primarily responsible for the effect produced by a chemical product.
Acute	Having rapid onset and of short duration.
Carcinogenicity	The ability to cause cancer.
Chronic	Of long duration.
Codex MRL	Internationally published standard maximum residue limit.
Desorption	Removal of an absorbed material from a surface.
Efficacy	Production of the desired effect.
Formulation	A combination of both active and inactive constituents to form the end use product.
Genotoxicity	The ability to damage genetic material
Hydrophobic	Water repelling
Leaching	Removal of a compound by use of a solvent.
Log P_{ow}	Log to base 10 of octonol water partitioning co-efficient.
Metabolism	The conversion of food into energy
Photodegradation	Breakdown of chemicals due to the action of light.
Photolysis	Breakdown of chemicals due to the action of light.
Subcutaneous	Under the skin
Toxicokinetics	The study of the movement of toxins through the body.
Toxicology	The study of the nature and effects of poisons.

References

- Australian Pesticides and Veterinary Medicines Authority 1996, *Ag Manual: The Requirements Manual for Agricultural Chemicals*, APVMA, Canberra.
- Australian Pesticides and Veterinary Medicines Authority 1997, *Ag Requirements Series: Guidelines for Registering Agricultural Chemicals*, APVMA, Canberra. (See footnote below).
- Australian Pesticides and Veterinary Medicines Authority 1996, *MRL Standard: Maximum Residue Limits in Food and Animal Feedstuffs*, APVMA, Canberra. (See footnote below).
- Australian Pesticides and Veterinary Medicines Authority 2001, *Ag Labelling Code—Code of Practice for Labelling Agricultural Chemical Products*, APVMA, Canberra. (See footnote below).
- Baumann, P., Clark, M.A., Baumann, L. and Broadwell, A.H. (1991) *Bacillus sphaericus* as a mosquito pathogen-properties of the organism and its toxins. *Microbiology Review*. 55:425-436.
- Charles, J.F. and Nielsen-LeRoux, C. (2000) Mosquitocidal bacterial toxins: diversity, mode of action and resistance phenomena. *Memorias do Instituto Oswaldo Cruz*. 95 (Suppl.1):201-206.
- Mulla, M.S., Thavara, U., Tawatsin, A., Chomposri, J. and Su, T. (2003) Emergence of resistance and resistance management in field populations of tropical *Culex quinquefasciatus* to the microbial control agent *Bacillus sphaericus*. *Journal of the American Mosquito Control Association*. 19(1):39-46.
- Priest, F.G., Ebdrup, L., Zahner, V. and Carter, P.E. (1997) Distribution and characterisation of mosquitocidal toxin genes in some strains of *Bacillus sphaericus*. *Applied Environmental Microbiology*. 63(4), 1195-1198.
- Regis, L., de Silva, S. and Melo-Santas, M. (2000) The use of mosquito and black fly control programs in Brazil. *Memorias do Instituto Oswaldo Cruz*. 95 (Suppl. 1):207-210.
- Servant, P., Rasso, M.L., Hamon, S., Poncet, S., Delcluse, A., Rapoport, G. (1999) Production of Cry11A and Cry11Ba toxins in *Bacillus sphaericus* confers toxicity towards *Aedes aegypti* and resistant *Culex* populations. *Applied and Environmental Microbiology* 65(7), 3021-3026
- Zahiri, N.S., Su, T. and Mulla, M.S. (2002) Strategies for the management of resistance in mosquitoes to the microbial control agent *Bacillus sphaericus*. *Journal of Medical Entomology*. 39(3):513-520.

Footnote:

Updated versions of these documents are available on the APVMA website
<http://www.APVMA.gov.au>.

APVMA PUBLICATIONS ORDER FORM

To receive a copy of the full technical report for the evaluation of *Bacillus sphaericus* Strain 2362 in the product VectoLex WG Larvicide, please fill in this form and send it, along with payment of \$30 to:

David Hutchison
Pesticides Division
Australian Pesticides and Veterinary Medicines Authority
PO Box E240
Kingston ACT 2604

Alternatively, fax this form, along with your credit card details to:
David Hutchison, Pesticides Division at (02) 6272 3218.

Name (Mr, Mrs, Ms, Dr) _____
Position _____
Company/organisation _____
Address _____
Contact phone number (____) _____

I enclose payment by cheque, money order or credit card for \$_____

Make cheques payable to 'Australian Pesticides and Veterinary Medicines Authority'.

Bankcard Visa Mastercard

Card number ____/____/____/____ Expiry date/...../.....

Signature _____ Date _____

[blank page here]