



Australian Government
**Australian Pesticides and
Veterinary Medicines Authority**



PUBLIC RELEASE SUMMARY

on the evaluation of the new active *Bacillus amyloliquefaciens* strain QST 713
in the product Serenade Opti Biofungicide

APVMA product number 82242

DECEMBER 2017

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ISBN: 978-1-925390-94-0

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PREFACE

The Australian Pesticides and Veterinary Medicines Authority (APVMA) is the Australian Government regulator 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 the Environment and Energy and State Departments of Primary Industries.

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 products containing new active constituents.

The information and technical data required by the APVMA to assess the safety of new chemical products, and the methods of assessment, must be consistent with accepted scientific principles and processes. Details are outlined on the [APVMA website](#).

This Public Release Summary is intended as a brief overview of the assessment that has been conducted by the APVMA and of the specialist advice received from 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.

About this document

This is a Public Release Summary.

It indicates that the Australian Pesticides and Veterinary Medicines Authority (APVMA) is considering an application for registration of an agricultural or veterinary chemical. It provides a summary of the APVMA's assessment, which may include details of:

- the toxicology of both the active constituent and product
- the residues and trade assessment
- occupational exposure aspects
- environmental fate, toxicity, potential exposure and hazard
- efficacy and target crop or animal safety.

Comment is sought from interested stakeholders on the information contained within this document.

Making a submission

In accordance with sections 12 and 13 of the Agvet Code, the APVMA invites any person to submit a relevant written submission as to whether the application for approval of the new active constituent and registration of Serenade Opti Biofungicide should be granted. Submissions should relate only to matters that the APVMA is required, by legislation, to take into account in deciding whether to grant the application. These matters include aspects of public health, occupational health and safety, chemistry and manufacture, residues in food, environmental safety, trade, and efficacy and target crop or animal safety. Submissions should state the grounds on which they are based. Comments received that address issues outside the relevant matters cannot be considered by the APVMA.

Submissions must be received by the APVMA by close of business on 9 January 2018 and be directed to the contact listed below. All submissions to the APVMA will be acknowledged in writing via email or by post.

Relevant comments 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.

When making a submission please include:

- contact name
- company or group name (if relevant)
- email or postal address (if available)
- the date you made the submission.

All personal information, and confidential information judged by the APVMA to be confidential commercial information (CCI)¹ contained in submissions will be treated confidentially.

Written submissions on the APVMA's proposal to grant the application for registration that relate to the grounds for registration should be addressed in writing to:

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¹ A full definition of 'confidential commercial information' is contained in the Agvet Code.

Further information

Further information can be obtained via the contact details provided above.

Further information on Public Release Summaries can be found on the APVMA website: www.apvma.gov.au

1 INTRODUCTION

1.1 Applicant

Bayer Cropscience PTY LTD.

1.2 Purpose of application

Bayer Cropscience Pty Ltd has applied to the APVMA for registration of the new product Serenade Opti Biofungicide containing not less than 1.3×10^{10} colony forming units (CFU) per gram of the new active constituent *Bacillus amyloliquefaciens* strain QST 713 as a wettable powder formulation. Serenade Opti Biofungicide contains a strain of bacterium originally identified as *B. subtilis* strain QST 713 as the active constituent. Recent publications and advances based on phylogenetic analysis and new taxonomic studies (Riboprinter system) have established the bacterium to be more accurately characterised as *B. amyloliquefaciens* strain QST 713. For registration purposes the APVMA proposes to register Serenade Opti Biofungicide containing not less than 1.3×10^{10} CFU/g of the new active constituent *B. amyloliquefaciens* strain QST 713.

This publication provides a summary of the information reviewed and an outline of the regulatory considerations for the proposed registration of Serenade Opti Biofungicide, and approval of the new active constituent, *B. amyloliquefaciens* strain QST 713.

1.3 Product claims and use pattern

Serenade Opti Biofungicide is a biological fungicide intended for use as a preventative treatment in the control of botrytis bunch rot in grapevines. It is applied at a rate of 250 g/100 L with applications made at flowering, pre bunch closure and veraison through to harvest.

1.4 Mode of action

The bacterium, *B. amyloliquefaciens*, is prevalent in soils and has been found in a variety of habitats worldwide. The QST 713 strain of *B. amyloliquefaciens* is known to be antagonistic toward fungal plant pathogens. This antagonism may be achieved in several ways including nutrient competition, site exclusion, colonisation, and attachment of the bacteria to the fungal pathogen.

The Fungicide Resistance Action Committee (FRAC), has designated *B. amyloliquefaciens* strain QST 713 (synonym *B. subtilis* strain QST 713) as a Group 44 fungicide, the mode of action has been defined as being a microbial disrupter of pathogen cell membranes.

1.5 Overseas registrations

The active constituent *B. amyloliquifaciens* strain QST 713 was first registered with the United States Environmental Protection Authority (US EPA) in June 2000 as *B. subtilis* strain QST 713.

The original registration of Serenade Opti refers to the active constituent *Bacillus subtilis* strain QST 713 as it was originally characterised. Serenade Opti containing the active constituent *B. subtilis* strain QST 713 is currently registered in the US, Canada, New Zealand and Mexico for a host of crops including brassica vegetables, bulb vegetables, berries, citrus, pome fruit, stone fruit, cucurbits, fruiting vegetables, leafy vegetables, grapes and many other crop types. Serenade Opti is registered as a broad spectrum, preventative product for the control or suppression of many important plant diseases including *Sclerotinia sp.*, *Botrytis sp.*, *Alternaria sp.*, *Venturia sp.* and various other pathogens of economic importance.

2 CHEMISTRY AND MANUFACTURE

2.1 Active constituent

Bacillus amyloliquefaciens QST 713 is a bio-fungicide for agricultural use.

B. amyloliquefaciens QST 713 is a Gram-positive, motile rod measuring 1.0 µm × 3-5 µm with peritrichous flagella, and is differentiated from other *Bacillus* species using ribotyping. The strain *B. amyloliquefaciens* QST 713 was originally isolated from soil in a private peach tree orchard in Fresno County, California (USA) in 1995.

The active constituent is identified as below:

COMMON NAME:	<i>Bacillus amyloliquefaciens</i> QST 713
SPECIES NAME:	<i>Bacillus amyloliquefaciens</i> (previously known as <i>Bacillus subtilis</i>)
STRAIN NAME:	QST 713
GENUS NAME:	<i>Bacillus</i>
FAMILY NAME:	<i>Bacillaceae</i>
GROUP:	Gram-positive eubacteria
KINGDOM:	Bacteria

Bacillus amyloliquefaciens QST 713 is not isolated as an active constituent during production of the agricultural product Serenade Opti Biofungicide. The microorganism is fermented and formulated into the product in a continuous process. However, the whole broth, at the time-point at which QC samples are taken, prior to concentration of the whole broth (if required) and/or addition of other ingredients to create formulated products (in this case Serenade Opti Biofungicide) is defined as an active constituent for assessment purposes.

The APVMA has evaluated the chemistry aspects of *B. amyloliquefaciens* QST 713 (identification, stability, manufacturing process, quality control procedures, batch analysis results and analytical methods) and found them to be acceptable.

Based on a review of the data provided by the applicant, the APVMA is satisfied that the chemistry and manufacturing details of *B. amyloliquefaciens* QST 713 are acceptable.

2.2 Formulated product

The product Serenade Opti Biofungicide will be manufactured overseas and imported into Australia in 0.1 kg – 100 kg polypropylene bottles and multilayer bags (including polypropylene or polyethylene bags).

The APVMA has evaluated the chemistry aspects of Serenade Opti Biofungicide (stability, formulation, manufacturing process, quality control procedures, specifications, batch analysis results, stability, analytical methods, packaging, and the chemistry aspects of the proposed label) and found them to be acceptable.

Serenade opti biofungicide

DISTINGUISHING NAME:	Serenade Opti Biofungicide
FORMULATION TYPE:	Wettable Powder (WP)
ACTIVE CONSTITUENT CONCENTRATION:	1.3×10^{10} CFU/g <i>Bacillus amyloliquefaciens</i> strain QST 713

Physical and chemical properties of the formulated product

PHYSICAL FORM:	Solid powder
COLOUR:	Light brown
ODOUR:	Yeast-like
PH (1% SOLUTION):	6.38
PACK SIZES AND MATERIALS:	100 g – 100 kg polypropylene bottles and multilayer bags (including polypropylene or polyethylene bags)
PRODUCT STABILITY:	Product will remain stable for 36 months when stored at ambient conditions (17–27°C) and 30 months when stored at 40°C

2.3 Recommendations

Based on a review of the chemistry and manufacturing details provided by the applicant, the registration of Serenade Opti Biofungicide, and approval of the active constituent *B. amyloliquefaciens* QST 713, are supported from a chemistry perspective.

3 TOXICOLOGICAL ASSESSMENT

3.1 Evaluation of toxicology

The toxicological database for *B. amyloliquifaciens* QST 713 and the product Serenade Opti Biofungicide, containing 1.3×10^{10} CFU/g *B. amyloliquifaciens* QST 713, was considered adequate to determine its toxicology profile and to characterise the risk to humans.

The toxicological database for *B. amyloliquifaciens* QST 713 consisted of toxicity tests conducted in laboratory animals. 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.

Chemical class

No pharmacokinetic studies were available for *B. amyloliquifaciens*, QST 713. *B. amyloliquifaciens* QST 713 is an environmental organism, commonly and normally present on fruit and vegetables. Ingested organisms from residues on treated produce will be subject to the normal digestive and immune surveillance mechanisms applicable to any ingested organism. The organism does not produce toxins or antimicrobial compounds under the culture conditions used for production. Consequently, pharmacokinetic studies are neither appropriate nor necessary.

Acute toxicity

The acute toxicity of *B. amyloliquifaciens* QST 713 was low by oral, dermal and intra-tracheal administration ($LD_{50} > 1.13 \times 10^8$ CFU/rat, > 2000 mg/kg bw and $> 1.2 \times 10^8$ CFU/rat respectively). *Bacillus amyloliquifaciens* QST 713 was a slight eye irritant but not a skin irritant.

Acute microbial pesticide evaluation also included an acute intravenous toxicity study for infectivity where the intravenous LD_{50} was $> 9.4 \times 10^6$ CFU/rat, demonstrating low systemic infectivity/pathogenicity potential (in rodents). No treatment-related mortalities occurred in any of the acute toxicity studies.

The acute toxicity profile for the product, Serenade Opti Biofungicide, was low by oral, dermal and inhalation routes of administration. The product was not a skin irritant or a skin sensitiser. However, the product was a severe eye irritant in rabbits.

Repeat-dose toxicity

Repeat dose toxicity studies were not conducted based on the absence of findings in the acute toxicology and infectivity/pathogenicity studies. This is reasonable and appropriate for a non-pathogenic biological active ingredient of this type. A 4-week inhalation study was performed in rats in which no toxicologically significant adverse effects were observed at the single dose level tested.

Supportive evidence for minimal short-term toxicity was provided by a 28-day oral toxicity of surfactin C (a surfactant produced by *B. amyloliquefaciens*) in rats. No clinical signs of toxicity, alterations in body weight, body weight gain and food consumption, changes in the haematological parameters, differences in organ weights or histopathological findings were noted at 500 mg/kg bw/d. Decreased bodyweight, increased alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase, increased liver weights and hydropic necrosis of hepatocytes were observed at higher doses.

Supportive evidence for the absence of potential genotoxicity, reproductive toxicity and developmental toxicity in rats was presented in a published study on surfactin C.

Clinical observations

A review of the safety concerns for the Qualified Presumption of Safety (QPS) status of biological agents that are added intentionally to food and animal feeds including *Bacillus* species was conducted by the European Food Safety Authority (EFSA 2013). The review addressed reports of claims where *B. amyloliquefaciens* was the causative agent in a range of clinical reports in workers engaged in the manufacture of the active constituent or formulation of the product. No evidence was found to indicate that *B. amyloliquefaciens* in general, or *B. amyloliquefaciens* QST 713 in particular, was the causative agent.

3.2 Public health standards

Poisons standard

On 31 October 2017 the Delegate of the Secretary of the Department of Health published a final Scheduling decision to exempt *B. amyloliquefaciens* QST 713 from Scheduling in the Poisons Standard. A cross reference in the Poisons Standard to *Bacillus subtilis* QST 713 for this organism will also be made. An implementation date of 1 February 2018 was notified for this decision.

Health-based guidance values

The Acceptable Daily Intake (ADI) is that quantity of a compound that can safely be consumed on a daily basis for a lifetime. The Acute Reference Dose (ARfD) is the maximum quantity of a compound that can safely be consumed over a short period of time, usually in one meal or during one day. As *B. amyloliquefaciens* QST 713 is a common environmental organism, and was not found to be infective or pathogenic at limit doses in acute toxicity tests, the establishment of an ADI or an ARfD was not required.

4 RESIDUES ASSESSMENT

4.1 Metabolism

No metabolism studies are available for *B. amyloliquefaciens* QST 713 in target plants or animals. However *B. amyloliquefaciens* QST 713 is a naturally occurring organism which does not produce a mammalian toxin or toxic metabolite. No kinetics and metabolism data are required for this microbial product.

4.2 Residues in foods and animal feeds

Both *B. amyloliquefaciens* and *B. subtilis* are commonly occurring soil bacterium and *B. subtilis* is registered for use as a direct fed microbial product in animals. A Table 5 entry covering use of *B. subtilis* as a direct fed microbial is established. Table 5 lists uses of substances where MRLs are not necessary. MRLs are not necessary in situations where residues do not or should not occur in foods or animal feeds; or where the residues are identical to or indistinguishable from natural food components; or otherwise are of no toxicological significance.

Given the ubiquity of the bacterium in the soil and other environments and the lack of any identified toxicological hazard (as confirmed by the APVMA toxicology assessment), the proposed use of *B. amyloliquefaciens* QST 713 in grapes for control of botrytis bunch rot is not expected to pose a risk to the health of people consuming treated grapes. A Table 5 entry in the MRL Standard for *B. amyloliquefaciens* QST 713 when used as a fungicide on food producing crops is appropriate to cover the proposed use on grapes.

4.3 Animal commodity MRLs

Grape pomace from wine or juice production from treated crops may be fed to livestock. Treated vineyards may be grazed by cattle or sheep and it is noted that a grazing WHP or restriction has not been proposed, but are not considered to be necessary.

It is noted that there is already a Table 5 entry for *B. subtilis* for use as a direct fed microbial in animals. Feeding of treated pomace or grazing of treated vineyards to livestock is therefore not expected to be an issue from a residues perspective.

4.4 Dietary risk assessment

A Table 5 entry to the MRL Standard has been recommended to cover the proposed use. The APVMA toxicology assessment concluded that health based guidance values were not necessary. It is therefore not necessary to undertake a dietary risk assessment.

4.5 Recommendations

In considering the application, the following amendments will be made to the APVMA MRL standard:

Table 5

SUBSTANCE	USE
Bacillus subtilis	
DELETE:	
	{T} When used on grapes
ADD:	
Bacillus subtilis strain QST 713 (Bacillus amyloliquefaciens)	When used as a fungicide on food producing crops

5 ASSESSMENT OF OVERSEAS TRADE ASPECTS OF RESIDUES IN FOOD

B. amyloliquefaciens QST 713 is ubiquitous in the environment. Detectable pesticide residues are not expected to be found in grapes as a result of the proposed use. As a result there is not expected to be any risk to trade of grapes or wine.

6 OCCUPATIONAL HEALTH AND SAFETY ASSESSMENT

6.1 Use pattern

Serenade Opti Biofungicide is formulated as a wettable powder (1.3×10^{10} CFU/gram *B. amyloliquefaciens* QST 713) and diluted at a rate of 250 g/100 L water for application. The diluted product is to be applied until foliage run-off. It is anticipated that up to 10 applications of Serenade Opti Biofungicide may be made annually; however, this maximum value is not specified on the product label. The diluted product is to be applied using radial fan, air shear and airblast machinery. The estimated area treated per day ranged 8–48 hectares (ha).

6.2 Exposure during use

Serenade Opti Biofungicide is for professional use only (farmers and commercial spray operators). Workers may be exposed to the product when opening containers, using the product, cleaning up spills, maintaining equipment and entering treated areas. The main routes of exposure to the product will be dermal and inhalation. The pattern of exposure was considered to be long-term based on multiple applications throughout the year as part of a preventative fungicide program.

A no observed adverse effect level (NOAEL) for occupational health and safety assessment purposes was not considered necessary because no long-term adverse effects from ingestion were anticipated with the microbial preparation. Instead, the occupational exposure and risk assessment were considered in terms of likely exposure compared to acute hazards identified in the acute toxicity studies and the possibility of sensitisation arising from pulmonary exposure to *B. amyloliquefaciens* QST 713.

6.3 Exposure during re-entry

The risk associated with re-entering treated areas is expected to be limited to exposure via the dermal route; exposure to dried spray may occur with activities such as the inspection of treated plants.

Given that no long-term adverse effects from ingestion were anticipated with the microbial preparation, a quantitative risk assessment of post-application exposure was not considered necessary. The risk to workers re-entering treated crops was considered to be low, and therefore no-entry interval was considered necessary.

6.4 Recommendations for safe use

The following first aid instructions and safety directions are recommended for inclusion on the product label:

First aid instructions

If poisoning occurs, contact a doctor or Poisons Information Centre. Phone Australia 131126; New Zealand 0800 764 766.

Safety directions

Harmful if inhaled. Will irritate the eyes. Repeated exposure may cause allergic disorders. Do not inhale dust or spray mist. Avoid contact with the eyes. When opening the container and preparing the spray wear cotton overalls buttoned to the neck and wrist, disposable dust face mask covering mouth and nose and disposable gloves. If product in eyes, wash it out immediately with water. Wash hands after use. After each day's use wash contaminated clothing.

6.5 Conclusion

The registration of Serenade Opti Biofungicide, containing 1.3×10^{10} CFU/g of *B. amyloliquefaciens* QST 713 in a wettable powder formulation as a fungicide in agricultural and horticultural crops, is supported from a human health perspective.

7 ENVIRONMENTAL ASSESSMENT

7.1 Introduction

Serenade Opti Biofungicide is a wettable powder for the biological control of botrytis bunch rot in grapevines. The product contains *B. amyloliquefaciens strain QST 713*.

The designated rate of use for Serenade Opti Biofungicide is 250 g/100 L of water, and a maximum of 1500 L will be sprayed per hectare (3.75 kg/ha).

Environmental fate and effects studies were provided in support of the application, and this information was sufficient to undertake an environmental risk assessment.

B. amyloliquefaciens is a common soil bacterium which occurs on all continents. The microorganism is able to form spores that can survive for long periods of time, and spores can be found in soil, water and air

7.2 Fate and environmental concentrations

The fate and behaviour of the microorganism in the environment is dependent upon a range of factors, including competition with other microorganisms (parasites/predators), soil parameters (pH, moisture, clay content) and agricultural practices such as tillage. The predicted environmental concentrations of the strain in soil and water after a single foliar application were calculated to be 2.3×10^5 CFU/g and 1.1×10^8 CFU/L, respectively, and after 10 applications, 2.3×10^6 CFU/g and 1.1×10^9 CFU/L, respectively. Although the level of the bacterium may fluctuate, both within a certain area and between areas, review of the submitted studies and literature suggest that the level of the microorganism that is applied to the environment will likely decrease over time, with DT₅₀ values for vegetative cells in both soil and water being only a few days, while endospores levels will likely be more stable.

B. amyloliquefaciens could be potentially spread by the movement of surface water, particularly rainfall, floods, spray drift and run-off. However, water is not regarded as being optimal for its survival as it is usually low in organic carbon. The bacterium could be spread by air as an endospore, which can be blown in the wind and carried on particles of dust.

7.3 Environmental effects

Biological pesticides can have direct and indirect adverse effects on non-target organisms, through a range of mechanisms including the production of a toxic secondary metabolites or pathogenesis.

The mode of action for suppression of botrytis bunch rot by this product is unclear. *B. amyloliquefaciens* produces a range of peptides and other secondary metabolites. Some of these metabolites have antimicrobial activity, however the mechanism of suppression could also be competition or providing a barrier to infection.

A discussion of potential effects on non-target-organisms is included in the risk assessment below.

7.4 Risk assessment

The risk assessment evaluated the potential adverse effects of *B. amyloliquefaciens* QST 713 to non-target organisms.

Mammals

No mammalian studies were submitted as part of the environmental package. However, available studies in international assessments indicate that *B. amyloliquefaciens* QST 713 is non-toxic to mammals. The lowest LD₅₀ value was LD₅₀ > 9.4 x 10⁶ CFU/animal for the proposed application rate. Although some infections of humans by the closely related species *B. subtilis* have been recorded, most have occurred in patients who are immunologically compromised or are related to drug abuse. The risk to mammals is considered acceptable.

Birds, reptiles and amphibians

Three studies were submitted that examined the effects of *B. amyloliquefaciens* or *B. subtilis* on birds. Two of these examined the use of *B. amyloliquefaciens* or *B. subtilis* as a probiotic in turkeys and chickens, both showing that the bacterium had no adverse effects on these birds. The third study examined the effects of a cottonseed diet that had been fermented with *B. subtilis* on chickens, concluding there were no adverse effects. It is noted that *B. amyloliquefaciens* or *B. subtilis* strains are regularly used as probiotics for birds. No study was presented investigating effects on amphibians and/or reptiles. Consultation of publicly available literature failed to find any reference recording *B. subtilis* as a pathogen of these organisms. The risk to birds, reptiles and amphibians is considered acceptable.

Bees

Ten studies were submitted that involve either the honeybee or the bumblebee, most involving the *B. amyloliquefaciens* strain QST 713. Of particular note is one report that reviews four studies on honeybees that were submitted to the US EPA in regards to the registration of Serenade WP Biofungicide in the United States. The LC₅₀ reported in this review from a 48 hour contact toxicity test was 200 µg product/bee, which is considered to be 'very slightly toxic/practically non-toxic' to bees. The risk assessment determined the risk quotient (RQ) value of 0.045. Therefore, the risk to bees is considered acceptable.

Arthropods and nematodes

Studies were presented on the toxicity of *B. amyloliquefaciens* or *B. subtilis* on the parasitic wasp (*Nasonia vitripennis*), ladybird (*Hippodamia convergens*), green lacewing (*Chrysoperla carnea*) larvae, predatory mite (*Typhlodromos pyri*), aphid parasitoid (*Aphidius rhopalosiph*), kola nut weevil (*Balanogastriis kolae*), velvetbean caterpillar (*Anticarsia gemmatilis*), wireworm (*Agriotes lineatus*), and a number of nematodes. Endpoints were provided for the parasitic wasp, ladybird, and larvae of the green lacewing. Using a LR₅₀ value of 12,120 g/ha for the parasitic wasp, a hazard quotient (HQ) of 1.08 was calculated, this value indicating a low risk to arthropods. No evidence could be found in the publicly available literature that *B. amyloliquefaciens* QST 713 or *B. subtilis* is a pathogen of invertebrates or nematodes. The risk to arthropods and nematodes is considered acceptable.

Earthworms

An acute toxicity study of *B. amyloliquefaciens* QST 713 with the earthworm *Eisenia fetida* gave a LC₅₀ of > 1000 mg test substance/kg dry soil. On the basis of toxicity rankings, the fungus is “very slightly toxic” to earthworms. Earthworms appear to be extremely resistant to pathogens, there being no recorded microbial pathogen of these organisms that has detrimental effects on their growth and survival. The assessment determined that the risk to earthworms is acceptable.

Microorganisms

B. amyloliquefaciens strain QST 713 is to be used as an agent to control *Botrytis cinerea* on grapevines. As such the bacterium is expected to have adverse effects upon the target fungus, and very likely other foliar microorganisms. Four studies examined the effects that strains of *Bacillus* species (including *B. amyloliquefaciens* and *B. subtilis*) had upon microorganisms. Two studies showed that strains of *B. amyloliquefaciens* and *B. subtilis* could have activity against a range of fungi and although non-target effects of these organisms have been observed, they have usually been small and transient and are unlikely to lead to a significant adverse indirect effect upon other aspects of an ecosystem, including non-target organisms of other taxa (eg plants, higher animals) that may be dependent upon fungi or microorganisms for food. The other studies demonstrated that *Bacillus* species had minimal effects on microbial communities. The risk to microorganisms is considered acceptable.

Aquatic organisms

Ten aquatic organism effects studies were submitted by the applicant. Non quantitative observations provide evidence that *B. amyloliquefaciens* QST 713 has only minimal effects upon aquatic organisms. Necropsy of rainbow trout and grass shrimp exposed to this bacterium failed to show any signs of infection of organs. The most sensitive endpoints came from chronic studies: EC₅₀ of 1.6 x 10⁶ CFU/mL and 21 day NOEC of 7.9 x 10⁵ CFU/mL for *Daphnia*; and LC₅₀ of 1.4 x 10⁷ CFU/mL for rainbow trout. It is notable that in these studies the mortality from exposure to the test organism was similar to the mortality of the attenuated (heat-killed) control, suggesting that mortality was due to the physical nature of the bacterial material, rather than its biological activity. It was concluded that the risk to aquatic organisms is acceptable under field conditions.

Terrestrial plants

Bacillus amyloliquefaciens has not been recorded as a phytopathogen, and therefore, adverse effects upon plants is not expected. The risk to terrestrial plants is considered acceptable.

7.5 Conclusions

The environmental risk assessment was based on the submitted data and review of publically available literature. The risks of the active constituent to terrestrial vertebrates, bees, arthropods, nematodes, earthworms, microorganisms and plants are considered acceptable.

The assessment showed that the risk to aquatic organisms under real field conditions is acceptable. The APVMA is unaware of any report of a significant adverse effect on a non-target organism by any strain of *B. amyloliquefaciens* or *B. subtilis* that has been used as a biological control agent.

The APVMA is satisfied that the proposed use of this product is unlikely to have an unintended effect that is harmful to animals, plants or things or the environment.

8 EFFICACY AND SAFETY ASSESSMENT

8.1 Proposed product use pattern

Serenade Opti Biofungicide is a biological fungicide intended for use as a preventative treatment in the control of botrytis bunch rot in grapevines. It is applied at a rate of 250 g/100 L as a foliar spray with applications made at flowering, pre bunch closure and veraison through to harvest.

Application of Serenade Opti Biofungicide should be performed to the point of runoff, ensuring coverage of all foliage and fruit. It is anticipated that up to 10 applications of Serenade Opti Biofungicide may be made annually. A copy of the proposed label is included in section 9—Labelling Requirements.

8.2 Summary of evaluation of efficacy and crop safety

The applicant, Bayer Cropscience PTY LTD, presented results from eight Australian field trials (2013–2015) in South Australia, Victoria, Western Australia and Queensland that evaluated the efficacy and safety of *B. amyloliquefaciens* QST 713 and Serenade Opti Biofungicide against bunch rot in different varieties of botrytis susceptible wine grapes and one variety of table grapes in commercial vineyards under different disease pressures. Trials were designed to test efficacy against botrytis bunch rot infection, especially latent botrytis infection and also against post-veraison botrytis infection.

Serenade Opti Biofungicide was tested at the proposed label rate of 250 g/100 L as well as 125–600 g/100 L over two seasons in regions representative of Australian wine growing regions. Efficacy was assessed on natural disease infections in commercial vineyards. Assessments were made on the extent of botrytis infection in randomly selected grape bunches. Disease incidence and severity were determined. The product was applied in a preventative spray program with sprays of conventional industry fungicides for comparison.

The trials were randomised complete block designs, with four replicates and product rates equivalent to that proposed on the label as well as higher and lower rates. Spray volumes used were in the range of normal commercial practices. The trials were designed to test the efficacy of the product as a stand-alone product, an early season treatment and as a late season treatment, as part of a preventative program. In this way efficacy against latent botrytis and late season botrytis infections were tested.

Adequate controls and comparison with industry fungicides in each trial were included in the trials reported. The data were analysed appropriately. In all trials the treatments were replicated, statistical analyses were undertaken and assessments of crop safety at several stages throughout each of the trials were conducted.

Efficacy

For efficacy against botrytis bunch rot, eight field trials were submitted for evaluation where Serenade Opti Biofungicide was trialled as a full-season spray program with four to six sprays at key botrytis time points (10% capfall, 80% capfall, pre-bunch closure, veraison and harvest) at rates of 125, 250 and 500 g/100 L and compared to industry standards. Serenade Opti Biofungicide provided significant control of botrytis bunch rot at 250 g/100 L. Lower rates of 125 g/100 L were not effective in controlling botrytis bunch rot. There was no benefit in increasing the rate of Serenade Opti Biofungicide as the higher rate of 500 g/100 L was found to be as effective as 250 g/100 L, with similar levels of control reported at both rates.

For latent botrytis infection over flowering, four of the trials submitted used Serenade Opti Biofungicide sprays over flowering for control of latent infection. Sprays of Serenade Opti Biofungicide at 250 g/100 L were made at 10 and 80% capfall and pre-bunch closure with no post-veraison applications and compared with conventional fungicides or untreated controls. Serenade Opti Biofungicide provided significant control of latent botrytis infection at 250 g/100 L but was not as effective as a conventional spray program with approximately 42% control compared to 67% control, respectively.

For efficacy against botrytis infection post-veraison, six of the trials submitted used sprays of Serenade Opti Biofungicide at 125–250 g/100 L made from veraison to harvest and then compared with conventional fungicides or untreated controls. Serenade Opti Biofungicide provided significant control of botrytis at 250 g/100 L with 60% control equivalent to a conventional spray program and was effective in controlling late season botrytis infections.

The data submitted demonstrate that Serenade Opti Biofungicide and *B. amyloliquefaciens* strain QST 713 were efficacious in controlling botrytis bunch rot when used as part of a preventative spray program.

Crop safety

No phytotoxic symptoms were observed in any of the trials for grapes including table and wine varieties. The product was safe to use on grape leaves, flowers and berries prior to harvest of grapevine and table grapes.

Resistance management

The bacterium, *B. amyloliquefaciens*, is prevalent in soils and has been found in a variety of habitats worldwide. The QST 713 strain of *B. amyloliquefaciens* is known to be antagonistic toward fungal plant pathogens. This antagonism may be achieved in several ways including nutrient competition, site exclusion, colonisation, and attachment of the bacterium to the fungal pathogen.

The Fungicide Resistance Action Committee (FRAC), has designated *B. amyloliquefaciens* strain QST 713 as a Group 44 fungicide, the mode of action has been defined as being a microbial disrupter of pathogen cell membranes. The risk on the occurrence of development of resistance is thought to be low as the mode of action of strain QST 713 is achieved in several ways and does not rely solely on a single sit of action.

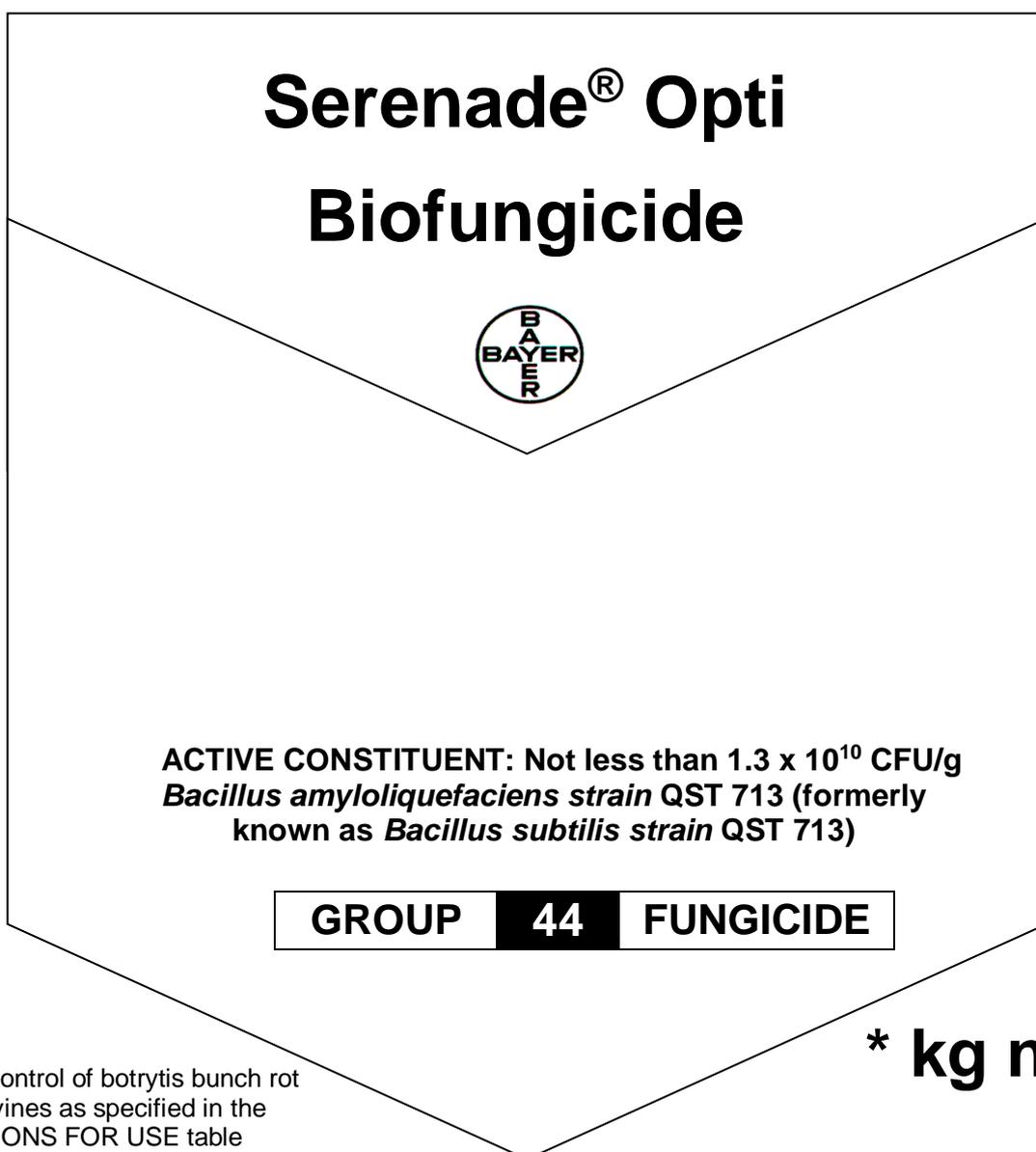
8.3 Conclusions

Trial data support that Serenade Opti Biofungicide will provide acceptable control against botrytis bunch rot on grapes when used as directed. Acceptable crop safety is expected when the product is used as directed. The directions for use are appropriate and consistent with fungicide use in commercial agriculture in Australia.

The application for registration of Serenade Opti Biofungicide is supported on efficacy and crop safety grounds when used in accordance with label directions.

9 LABELLING REQUIREMENTS

READ SAFETY DIRECTIONS BEFORE OPENING OR USING



*** kg net**

For the control of botrytis bunch rot in grapevines as specified in the DIRECTIONS FOR USE table

(Label code)

* 100 g – 100 kg

DIRECTIONS FOR USE**RESTRAINTS**

DO NOT apply with aircraft.

DO NOT apply when wind speed is less than 3 or more than 20 kilometres per hour as measured at the application site.

Users of this product **MUST make an accurate written record** of the details of each spray application within 24 hours following application and **KEEP** this record for a minimum of 2 years. The spray application details that must be recorded are: **1.** date with start and finish times of application; **2.** location address and paddock/s sprayed; **3.** full name of this product; **4.** amount of product used per hectare and number of hectares applied to; **5.** crop/situation and weed/pest; **6.** wind speed and direction during application; **7.** air temperature and relative humidity during application; **8.** nozzle brand, type, spray angle, nozzle capacity and spray system pressure measured during application; **9.** name and address of person applying this product. (Additional record details may be required by the State or Territory where this product is used.)

CROP	DISEASE/PEST	RATE	CRITICAL COMMENTS
Grapevines	Botrytis bunch rot	250 g/100 L water	<p>As part of a preventative spray program, applications may be made at, but not limited to, flowering, pre bunch closure, veraison through to harvest.</p> <p>Serenade Opti should not be applied to an existing disease infection.</p> <p>Spray to point of runoff, ensuring thorough coverage of all foliage and fruit.</p> <p>For concentrate spraying, do not use at rates greater than 2 times the dilute spraying rate (ie at a concentration factor greater than 2X) – refer “Application” section in GENERAL INSTRUCTIONS.</p>

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

WITHHOLDING PERIODS (WHP)

Grapevines: NOT REQUIRED WHEN USED AS DIRECTED

GENERAL INSTRUCTIONS

Serenade Opti has been developed from a naturally occurring strain of *Bacillus amyloliquefaciens* (formerly known as *Bacillus subtilis*). It is a broad spectrum preventative fungicide recommended for the control of botrytis in grapes. It acts by preventing spore germination, germ tube elongation and germ tube penetration. Serenade Opti can be integrated into an overall disease and pest management strategy wherever fungicide use is necessary in an IPM programme and is also an important tool in resistance management strategies. Serenade Opti should be applied prior to disease development, and should be part of a comprehensive botrytis management program.

Mixing

Partially fill the spray tank with clean water and start agitation. Add the specified amount of Serenade Opti for the area to be treated to the spray tank. Complete filling the tank to the required volume for the area to be treated. Maintain agitation until spraying is complete. Do NOT allow the spray mixture to stand overnight or for any prolonged periods. Spray pH must be not less than pH 4.5 and not greater than pH 8.5.

Application

Application should be by ground spray equipment only. Thorough coverage of the target area is essential.

Dilute spraying

- Use a sprayer designed to apply high spray volumes, up to the point of run-off and matched to the crop being sprayed.
- Set up and operate the sprayer to achieve even coverage throughout the crop canopy, including fruit. Apply sufficient water to cover the crop to the point of run-off. Avoid excessive run-off.
- The required spray volume may be determined by applying different test volumes, using different settings on the sprayer, or from industry guidelines or expert advice.
- Add the amount of product specified in the Direction for Use table for each 100 L of water. Spray to the point of run-off.
- The required dilute spray volume will change and the sprayer set up and operation may also need to be changed, as the crop grows.

Concentrate spraying

- Use a sprayer designed and set up for concentrate spraying (that is a sprayer which applies spray volumes less than those required to reach the point of run-off) and matched to the crop being sprayed.
- Set up and operate the sprayer to achieve even coverage throughout the crop canopy using your chosen spray volume.
- Determine an appropriate dilute spray volume (See *Dilute Spraying* above) for the crop canopy. This is needed to calculate the concentrate mixing rate.
- The mixing rate for concentrate spraying can then be calculated in the following way:

EXAMPLE ONLY

1. Dilute spray volume as determined above: For example 1500 L/ha
2. Your chosen concentrate spray volume: For example 750 L/ha
3. The concentration factor in this example is 2 X (ie $1500 \text{ L} \div 750 \text{ L} = 2$)
4. If the dilute label rate is 250 g/100 L, then the concentrate rate becomes 2 x 250 that is 500 g/100 L of concentrate spray.
 - The chosen spray volume, amount of product per 100 L of water, and the sprayer set up and operation may need to be changed as the crop grows.
 - Do not use at a concentration factor greater than 2X (eg at a rate higher than 500 g/100 L where a dilute spraying rate of 250 g/100 L is specified).
 - For further information on concentrate spraying, users are advised to consult relevant industry guidelines, undertake appropriate competency training and follow industry best practice.

Sprayer clean up

If clean-up of spray equipment is required, rinse equipment twice with clean water after use.

Visual residues on fruit

Visual residues may be left on the grapes from application of the product.

FUNGICIDE RESISTANCE WARNING

GROUP	44	FUNGICIDE
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Serenade Opti is a microbial fungicide. For fungicide resistance management the product is a Group 44 fungicide. Some naturally occurring individual fungi resistant to the product and other Group 44 fungicides may exist through normal genetic variability in any fungal population. The resistant individuals can eventually dominate the fungal population if these fungicides are used repeatedly. These resistant fungi will not be controlled by this product and other Group 44 fungicides, thus resulting in a reduction in efficacy and possible

yield loss. Since the occurrence of resistant fungi is difficult to detect prior to use, Bayer CropScience accepts no liability for any losses that result from failure of this product to control resistant fungi.

COMPATIBILITY

For the latest compatibility recommendations contact the Bayer CropScience Technical Information Service 1800 804 479 or your local Bayer CropScience representative.

STORAGE AND DISPOSAL

Store in closed original container in a dry, well ventilated place as cool as possible out of direct sunlight. Serenade Opti can be stored for 36 months from the date of manufacture when stored at ambient temperature conditions (17–27 °C).

Multilayer bag or multilayer bag in box (0.1–100 kg)

Single-rinse or shake remainder into spray tank. Do not dispose of undiluted chemicals on site. Puncture and deliver empty packaging to an approved waste management facility. If an approved waste management facility is not available, bury the empty packaging 500 mm below the surface in a disposal pit specifically marked and set up for this purpose, clear of waterways, desirable vegetation and tree roots, in compliance with relevant Local, State or Territory government regulations. Do not burn empty containers or product.

Polypropylene bottle (0.4–100 kg)

Triple-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 deliver empty packaging to an approved waste management facility. If an approved waste management facility is not available, bury the empty packaging 500 mm below the surface in a disposal pit specifically marked and set up for this purpose, clear of waterways, desirable vegetation and tree roots, in compliance with relevant Local, State or Territory government regulations. Do not burn empty containers or product. Do not use empty container for any other purpose.

SAFETY DIRECTIONS

Harmful if inhaled. Will irritate the eyes. Repeated exposure may cause allergic disorders. Do not inhale dust or spray mist. Avoid contact with the eyes. When opening the container and preparing the spray wear cotton overalls buttoned to the neck and wrist, disposable dust face mask covering mouth and nose and disposable gloves. If product in eyes, wash it out immediately with water. Wash hands after use. After each day's use wash contaminated clothing.

FIRST AID

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

SAFETY DATA SHEET

Additional information is listed in the Safety Data Sheet, which can be obtained from www.crop.bayer.com.au.

EXCLUSION OF LIABILITY

This product must be used strictly as directed, and in accordance with all instructions appearing on the label and in other reference material. So far as it is lawfully able to do so, Bayer CropScience Pty Ltd accepts no liability or responsibility for loss or damage arising from failure to follow such directions and instructions.

Serenade® is a Registered Trademark of the Bayer Group.

APVMA Approval No.: 82242/105270

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Technical Enquiries: 1800 804 479

FOR 24 HOUR SPECIALIST ADVICE IN EMERGENCY ONLY PHONE 1800 033 111
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Batch Number:

DOM:

Expiry date:

ABBREVIATIONS

ac	active constituent
ADI	Acceptable Daily Intake (for humans)
ai	active ingredient
ARfD	Acute Reference Dose
bw	bodyweight
°C	Degrees Celsius
CFU	Colony Forming Units
d	day
DAT	Days After Treatment
DT ₅₀	Time taken for 50% of the concentration to dissipate
EA	Environment Australia
E _b C ₅₀	concentration at which the biomass of 50% of the test population is impacted
EC ₅₀	concentration at which 50% of the test population are immobilised
EEC	Estimated Environmental Concentration
E _r C ₅₀	concentration at which the rate of growth of 50% of the test population is impacted
EI	Export Interval
ESI	Export Slaughter Interval
EUP	End Use Product
F ₀	original parent generation
g	gram
GAP	Good Agricultural Practice
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GVP	Good Veterinary Practice
h	hour
ha	hectare

HDPE	High Density Polyethylene
HPLC	High Pressure Liquid Chromatography or High Performance Liquid Chromatography
HQ	Hazard Quotient
id	intra-dermal
im	intra-muscular
ip	intra-peritoneal
IPM	Integrated Pest Management
iv	intra-venous
in vitro	outside the living body and in an artificial environment
in vivo	inside the living body of a plant or animal
kg	kilogram
K _{oc}	Organic carbon partitioning coefficient
L	Litre
LC ₅₀	concentration that kills 50% of the test population of organisms
LD ₅₀	dosage of chemical that kills 50% of the test population of organisms
LOD	Limit of Detection—level at which residues can be detected
LOQ	Limit of Quantitation—level at which residues can be quantified
LR ₅₀	Lethal rate that kills 50% of the test population of organisms
mg	milligram
mL	millilitre
MRL	Maximum Residue Limit
NDPSC	National Drugs and Poisons Schedule Committee
NEDI	National Estimated Daily Intake
NESTI	National Estimated Short Term Intake
ng	nanogram
NOEC/NOEL	No Observable Effect Concentration/Level
NOAEL	No Observed Adverse Effect Level

OC	Organic Carbon
OM	Organic Matter
po	oral
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
SDS	Safety Data Sheet
SUSMP	Standard for the Uniform Scheduling of Medicines and Poisons
TGA	Therapeutic Goods Administration
TGAC	Technical grade active constituent
T-Value	A value used to determine the First Aid Instructions for chemical products that contain two or more poisons
µg	microgram
WHP	Withholding Period
WP	Wettable Powder

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 a material from or through 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	repels water
Leaching	Removal of a compound by use of a solvent
Log Pow	Log to base 10 of octanol water partitioning co-efficient, synonym KOW
Metabolism	The chemical processes that maintain living organisms
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
Ubiquitous	Present, appearing or found everywhere

REFERENCES

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