Evaluation Report for Category B, Subcategory 2.1, 2.4, 3.11 Application

Application Number: 2014-1144
Application: New End-Use Product Chemistry – guarantee and proportion of formulants
New Product Labels – new pests
Product: Serenade Opti
Registration Number: 31666
Active ingredients (a.i.): *Bacillus subtilis* (strain QST 713)
PMRA Document Number : 2435725

Purpose of Application

The purpose of this application was to register a new product, Serenade Opti, containing *Bacillus subtilis* strain QST 713 (containing a minimum of 1.31 x 10^{10} CFU/g). This product is for use on a wide variety of fruits and vegetable crops.

Chemistry Assessment

The applicant submitted a description of the manufacturing process and the quality assurance protocol for Serenade Opti. Data were provided to confirm the product guarantee and to demonstrate that the microbial contaminants in the whole broth used to produce Serenade Opti are at acceptable levels. The submitted storage stability data were adequate to support a storage period of six years at room temperature.

Health Assessments

The active ingredient in Serenade Opti, *Bacillus subtilis* strain QST 713, was previously assessed for human health and safety and was found to be acceptable for registration.

Serenade Opti was found to be of low toxicity via the oral (LD_{50} >5000 mg/kg body weight (bw)), inhalation (LC_{50} >2.15 mg/L) and dermal (LD_{50} >5050 mg/kg bw) routes of exposure.

Although Serenade Opti caused irritation in a combined acute dermal toxicity and acute dermal irritation study, it must be noted that both the dose and period of exposure in this combined study were significantly higher and longer than that required for a dermal irritation study. Given that Serenade Opti is similar in formulation to Serenade MAX (Registration Number 28549), it is expected that, under the conditions of a standard acute dermal irritation study, Serenade Opti would exhibit a similar level of dermal irritancy. Serenade MAX was rated as being slightly irritating.

Although an eye irritation study resulted in a maximum average score (MAS) of 8.22 (average score from 24, 48 and 72 hour observations), the ocular irritation potential for Serenade Opti has been upgraded to mildly irritating as moderate to severe irritation was observed at the one hour

timepoint.

Serenade Opti is considered to be a sensitizer.

The use rates, application methods, and pest/crop combinations proposed for Serenade Opti are consistent or similar to those already permitted for the registered products Serenade MAX (Registration Number 28549) and Serenade ASO (Registration Number 28626). Therefore, apart from the requirement for eye goggles to be worn by individuals handling the product, no additional occupational exposure, bystander or food and feed residue concerns were identified. The label statements for Serenade Opti, coupled with its low toxicity, are considered adequate to address any potential risk due to occupational, bystander or dietary exposure.

Maximum Residue Limit

Bacillus subtilis strain QST 713 is currently registered in Canada. The establishment of a maximum residue limit (MRL) was not required for *B. subtilis* strain QST 713. Although it has been noted that some strains of *B. subtilis* have been isolated from food implicated in food poisoning, these strains demonstrated the ability to produce a highly heat-stable toxin that was not reported in *B. subtilis* strain QST 713. Furthermore, there was no significant toxicity and no signs of pathogenicity observed when *B. subtilis* strain QST 713 was administered orally to rats.

Incident Reports

As of September 2, 2014, there were no incident reports in the PMRA database for *B. subtilis* strain QST 713 or other *B. subtilis* strains.

A search of the California Pesticide Illness Database (1992 to 2010) identified five incidents involving 27 people related to *B. subtilis* strain QST 713. However, as each incident arose from occupational exposure following co-application with chemical pesticides and/or pheromones, causality could not equivocally be attributed to *B. subtilis* strain QST 713. Predominant symptoms included nausea, vomiting, headache, and respiratory, skin and eye irritation. These incident reports were considered in this evaluation and did not affect the risk assessment.

Environmental Assessment

The environmental database for *B. subtilis* strain QST 713 is complete and there are no outstanding environmental concerns for this active ingredient. As the use rates, application methods, and pest/crop combinations proposed for Serenade Opti are consistent or similar to those already permitted for the registered products Serenade MAX and Serenade ASO, no additional risks to the environment are expected.

Value Assessment

A total of 14 trials conducted on 10 different fruit and vegetable crops against 11 different pathogens were reviewed to support the registration. Bridging trials conducted on major crops and pests under low to high disease pressures clearly demonstrated that Serenade Opti and Serenade Max are biologically equivalent. All claims were extrapolated to the Serenade Opti label. Aerial application was supported for Crop Group 20A based on the precedent product.

Extrapolation of a claim for post-harvest application to potatoes from the Serenade ASO label was also supported based on biological equivalence that was previously demonstrated (ERC2007-06).

The registration of Serenade Opti provides an alternative to growers for suppression of a wide range of diseases and can be used in rotation with other chemical or biological fungicides. *Bacillus subtilis* strain QST 713 can be used as a resistance management tool due to its multiple-site mode of action and is also an option for organic vegetable production.

Conclusion

The Pest Management Regulatory Agency has completed an assessment of the information provided, and has found the information sufficient to support the registration of the new end-use product Serenade Opti.

Appendix I: Toxicity of Serenade Opti

Study Type	Species, Strain, and Doses	Result	Significant Effects and Comments	Reference(s)
Acute Toxicity of Serenade Opti				
Acute Oral Toxicity	Rat – Sprague-Dawley 3 ♀, 5000 mg/kg bw in deionized water (equivalent to a minimum of 6.55×10^{10} CFU/kg bw) by oral gavage	LD_{50} > 5000 mg/kg bw	-No mortalities, signs of toxicity or abnormalities upon necropsy. -All animals gained weight. **ACCEPTABLE** **LOW TOXICITY**	2409050
Acute Inhalation Toxicity	Rat – Sprague-Dawley 5/sex, 2.15 mg/L nose only for 4 hours	LC_{50} > 2.15 mg/L	-No mortalities. -Decreased activity observed in all test animals from 4.5 hour until up to three days after exposure. No other clinical signs observed. -Dark red lungs in one male rat; pale lungs in one female. -All animals gained weight. **ACCEPTABLE** **LOW TOXICITY**	2409052

Acute Dermal Toxicity/Acute Dermal Irritation	Rabbit – New Zealand White 5/sex, 5050 mg/kg bw moistened with deionized water, 24 hours	LD_{50} > 5050 mg/kg bw	-No signs of toxicity or adverse effects. -All animals, except one female, gained weight. -Pale lungs in one male and female rabbit. Dark spots on lungs of one male. No other abnormalities upon necropsy. **ACCEPTABLE for toxicity testing** **LOW TOXICITY** -Severe erythema in four animals and very slight erythema in remaining animals on Day 1. Severe erythema persisted in one animal throughout the 14-day study. Eschar formation, alopecia, ulceration, discolouration, coariaceousness and atonia also observed. -Exposure level and time higher and longer than recommended for assessment of dermal irritation. -Serenade MAX, an end-use product similar in formulation to Serenade Opti, was found to be slightly irritating [maximum irritation score (MIS) of 1.333 at the 30-60 minute timepoint]. Dermal irritation potential of Serenade Opti expected to be similar to that of Serenade MAX. **SUPPLEMENTAL for irritation testing** **SLIGHTLY IRRITATING**	2409054 1116597

Eye Irritation	Rabbit – New Zealand White 1 ♂; 2 ♀, 0.1 mL/animal	-MIS=45.33 -Maximum individual irritation score=57 at 1 hour timepoint -MAS=8.22 (24, 48, 72 hours)	-All animals exhibited redness (score=2), chemosis (score=2) and discharge (score=2) at the 1 hour timepoint. Corneal opacity (score=1 or 2) in greater than ¾ of the eye (score=4) was also observed in all animals at 1 hour. The irises of all treated eyes were also affected (score=1 or 2). -At the 24 hour timepoint, all animals continued to exhibit conjunctival redness (score=1 or 3), chemosis (score=1 to 3) and discharge (score=1). Corneal opacity was observed in only one animal (score=1) to greater than ¾ of the eye (score=4). The iris of one animal remained affected (score=1). -At the 48 hour timepoint, conjunctival redness (score=1 or 2) continued in all animals. Chemosis (score=2) was observed in one animal and discharge (score=1) in two animals. Slight dulling of the cornea was noted in one animal to less than ¼ of the eye (score=1). The iris of one animal remained affected (score=1). -All signs of ocular irritation were absent in all animals by the 72 hour timepoint. **MILDLY IRRITATING**	2409056

Sensitization	Guinea Pigs – Hartley-Albino -treatment group:10/sex -induction phase: 400 mg moistened with 0.4 mL deionized water for six hours; three applications total spaced weekly (Days 1, 8, 15) -challenge phase (Day 29): 400 mg moistened with 0.4 mL deionized water -naïve control group: 5/sex, untreated during induction phase, same challenge phase as above -historical positive control: 5/sex; 0.4 mL alpha-hexylcinnamaldehyde, tech. 85% for induction and challenge phases		-No dermal reactions noted after the first and second induction treatments (average score=0.0). After the third induction treatment, very faint and nonconfluent erythema (score=0.5) observed in four animals/sex. Faint and confluent (score=1) erythema noted in one male at this time. At 24 hours after the third induction, the average score=0.25. - At 24 and 48 hours after challenge, five ♂ and six ♀ animals in the treatment group exhibited very faint and nonconfluent erythema. A further three ♂ and two ♀ presented faint erythema. The average score amongst treatment group animals was 0.5 at 24 and 48 hours after challenge. -No dermal reactions noted in the naïve control group at 24 and 48 hours after challenge. -In the historical positive control group, the mean score of 1.2 compared to the negative control group score of 0.1 after challenge confirmed the sensitivity of guinea pigs. **POSITIVE SENSITIZER**	2409055

References

2409057 2014, Serenade Opti Biofungicide (*Bacillus subtilis* strain QST 713), DACO: M10.0, M10.1, M10.2, M10.2.1, M10.2.2, M10.3, M10.3.1

2409058 2014, Serenade Opti Biofungicide (*Bacillus subtilis* strain QST 713), DACO: M10.0, M10.1, M10.2, M10.2.1, M10.2.2, M10.3, M10.3.1, M10.4, M10.4.1, M10.4.2, M10.4.3, M10.4.4

2409060 2014, Serenade Opti Biofungicide (*Bacillus subtilis* strain QST 713) -Efficacy, DACO: M10.3

1117185 2003, Product Chemistry of Serenade MAX, DACO: 2.10, M2.7, M2.8, M2.9.2, M2.9.3 CBI

1643517 2008, Physico-chemical properties of the Formulation Serenade MAX over 2 Years at 20 degree C, DACO: M2.11 CBI

1906314	2009, 36 month - Storage stability testing, DACO: M2.11 CBI
1906316	2009, 68-Month Storage stability testing, DACO: M2.11 CBI
1906318	2009, 68 month - Storage stability testing, DACO: M2.11 CBI
2409045	2009, Product Chemistry, DACO: M2.12 CBI
2409046	2008, Five Batch Titer Analysis of Microbial Pesticide QRD 146, DACO: M2.10, M2.10.1, M2.8 CBI
1116597	1998, Primary Dermal Irritation Study in Rabbits with QST 713WP, DACO: M4.5.2
2409050	2008, Acute Oral Toxicity Study: Up & Down Procedure (UDP) in Rats, DACO: M4.2, M4.2.1, M4.2.2
2409052	2008, Acute Inhalation Toxicity Study in Rats, DACO: M4.2, M4.2.1, M4.2.3
2409054	2008, Acute Dermal Toxicity Study in Rabbits, DACO: M4.1, M4.4
2409055	2009, Skin Sensitization Study in Guinea Pigs, DACO: M4.1, M4.6
2409056	2008, Acute Eye Irritation Study Rabbits, DACO: M4.1, M4.9

ISSN: 1911-8082