Serenade ASO
(*Bacillus amyloliquefaciens* QST 713)
Microbial pest control product against plant pathogenic fungi and bacteria

Dossier according to OECD guidance for industry data submissions for microbial pest control products and their microbial pest control agents – August 2006

Summary documentation, Tier II

Annex IIIM1, Section 4

Point IIIM1 8: Rationale to waive residue studies on MPCP

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Applicant

Bayer CropScience AG

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OWNERSHIP STATEMENT

Introduction

The company Bayer CropScience AG is submitting a dossier for the re-approval of *Bacillus amyloliquefaciens* QST 713, previously designated as *Bacillus subtilis* QST 713, as an active substance under regulation (EC) 1107/2009. Due to changes in taxonomy, *B. subtilis* QST 713 is now classified as *B. amyloliquefaciens*. For further information, please refer to Annex II, Section 1, Point IIM 1.3.1 of this dossier. As a consequence, the active substance is now named *B. amyloliquefaciens* QST 713. The old strain designation is still used in some documents and can be considered as a synonym. Serenade ASO is the representative formulation for the process of the re-approval of *Bacillus amyloliquefaciens* QST 713 as an active substance under regulation (EC) 1107/2009.
Inclusion of *B. subtilis* QST 713 into Annex I of 91/414/EEC (now list of approved active substances according to (EU) No 540/2011) entered into force in February 2007 (Commission Directive 2007/6/EC). *B. subtilis* strain QST 713 was notified and defended by AgraQuest Inc. Although the formulation Serenade ASO was not the representative formulation in the dossier for Annex I inclusion of *B. subtilis* QST 713, here the data of the above mentioned product is summarized, since it represents latest information on *B. amyloliquefaciens* QST 713 formulation. The representative formulation for the initial Annex I inclusion, Serenade WP, is no longer produced.

Here we submit all studies reviewed on the zonal level and new data and information (public literature and summaries).
Critical Good Agricultural Practices for Serenade ASO are summarized in the table below.

Table 8-1 Summary of critical Good Agricultural Practice for Serenade ASO

Crop and/ or situation (crop destination / purpose of crop)	F G or I	Pests or Group of pests controlled	Application			Application rate			PHI (days)	Remarks
			Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	L product / ha a) max. rate per appl. b) max. total rate per crop/season	kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
Strawberry	G	*Botrytis cinerea*	Spraying	BBCH 55-89	a) 6 (5 days) b) 6 (5 days)	a) 10 b) 60	a) 0.140 kg min. 1x 10^{13} CFU/ha b) 0.84 kg min. 6 x 10^{13} CFU/ha	400-1000	n.r.	10 L/ha authorized in UK
Strawberry	F	*Botrytis cinerea*	Spraying	BBCH 55-89	a) 6 (5 days) b) 6 (5 days)	a) 8 b) 48	a) 0.112 kg min. 8 x 10^{12} CFU/ha b) 0.672 kg min. 4.8x 10^{13} CFU/ha	400-1000	n.r.	
Grapes	F	*Botrytis cinerea*	Spraying	BBCH 68-89	a) 9 (5 days) b) 9 (5 days)	a) 8 b) 72	a) 0.112 kg min. 8x 10^{12} CFU/ha b) 1.008 kg min. 7.2x 10^{13} CFU/ha	500-1000	n.r.	

n.r. – not relevant

IIIM1 8 Residues in/on food and feed products for the Microbial Pest Control Product (rationale to waive residue studies on MPCP)

This document reviews residue data for the microbial plant protection product Serenade ASO containing *Bacillus amyloliquefaciens* strain QST 713.

Serenade ASO is a biological fungicide formulated as suspension concentrate. The content of the active ingredient *B. amyloliquefaciens* QST 713 in Serenade ASO minimum content of viable spores of 1×10^{12} CFU/kg (or 1.0×10^{12} CFU/L).

With regard to safety issues, it is important to note that *B. subtilis* and *Bacillus amyloliquefaciens* are naturally present in our environment. Therefore, its application in control of plant pathogenic fungi means only a fluctuation of the bacterium population in the biotope of the target pathogen and does not disturb the natural micro-flora. The experience that *B. amyloliquefaciens* QST 713 presents no risk for humans, animals and the environment has been confirmed by numerous studies.

The applicant applies for a waiver for performing residue studies with Serenade ASO, based on the following considerations:

- Due to the fact that the active ingredient is a viable micro-organism of ubiquitous occurrence and predominance in the soil-microflora the term residue is not applicable to this preparation. Specifically, no residue metabolism can be stated, since a micro-organism does not follow first order kinetics. With regard to its natural global distribution and non-pathogenic character *B. subtilis* and *B. amyloliquefaciens* cells left on the surface of treated areas or plant products do not imply health or environmental impacts.

- No toxicity or pathogenicity was observed in an acute toxicity study in rats. Serenade ASO induced no signs of toxicity at a dose of 5000 mg/kg bw corresponding to at least 5×10^9 CFU per kg b.w. for male and female rats (please refer to Annex III Doc IIIM1 Section 3 Point 7.1.1).

- *Bacillus subtilis* and *B. amyloliquefaciens* occur ubiquitously and are one of the prevalent bacterial species in soils and on different plant surfaces. Colonization of different foodstuffs is common, but largely ignored because *B. subtilis* is generally accepted to be non-pathogenic.

- Following application of Serenade ASO, survival of the active substance *B. subtilis* on leaves and fruits is very limited. UV radiation is the major limiting factor for survival of bacteria on leaves. Environmental conditions are usually unfavourable and restrict microbial growth, thus explaining the generally low population levels of growing saprophytic bacteria on the leaf surface, e.g., *B. subtilis* and *B. amyloliquefaciens* cells will stop growth after depletion of organic matter supply. Several studies have shown a rapid decline of the bacterial populations a few days after application (please refer to Annex II Doc IIM Section 4 of the EU dossier of *B. subtilis* QST 713). Since colonization of the leaf surface by *B. amyloliquefaciens* contributes largely to the protective effect against bacterial and fungal pathogens, application of Serenade ASO has to be repeated frequently.

- *B. subtilis* has been used for enzyme production on a large industrial scale, and is even used for food production without having caused health or environmental hazards or damages.

- A plant product (fruit) carrying a layer built up of *Bacillus subtilis* or *B. amyloliquefaciens* can easily be washed with water prior to consumption or juice production.

Conclusion

Primarily the low health and environmental risk potential of *Bacillus subtilis* and *B. amyloliquefaciens*, and its ubiquitous distribution indicate that residual *Bacillus subtilis* and *B. amyloliquefaciens* cells may present only a low risk potential. Secondly, the unfavourable environmental conditions prevailing on the leaf and fruit surfaces and the dependence of *Bacillus subtilis* and *B. amyloliquefaciens* on organic matter supply are restricting its growth. In addition, in processing of raw products no growth or sporulation of *Bacillus subtilis* and *B. amyloliquefaciens* is expected to occur.

It has been concluded that following application of Serenade ASO according to GAP, no accumulation of *B. subtilis Bacillus amyloliquefaciens* QST 713 on leaves will occur since it was shown that persistence of *Bacillus subtilis* and *B. amyloliquefaciens* on leaves and fruit surfaces is low.

Serenade ASO contains the active substance *Bacillus amyloliquefaciens* strain QST 713. The Microbial Pest Control Agent *Bacillus subtilis* QST 713 was included into Annex I of Directive 91/414/EEC on 01.02.2007 and then approved according to the Commission Implementing Regulation (EU) No 540/2011 of 25.05.2011, implementing Regulation (EC) No 1107/2009 of the European Parliament. It was included under its old taxonomical name *Bacillus subtilis* QST 713. Due to the new information on *Bacillus* spp. taxonomy the strain QST 713 was found to belong to *Bacillus amyloliquefaciens* and therefore its name has been changed also in this dossier. For details on taxonomy please refer to Annex II, Section 1, Point IIM 1.3.1.