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 5

Point IIIM1 9, Fate and behaviour in the environment

Date: September 2015
Revised November 2015

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[1]). *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, Serenade WP, inclusion is no longer produced. Here we submit all studies and new data and information (public literature and summaries).

Critical Good Agricultural Practices for Serenade ASO are summarized in Table 9-1. These were used as reference for the calculation of exposure in the risk assessment. As worst case, the maximum number of applications was considered for the risk assessment within the frame of the risk envelope approach. Here we submit all new data and information based on previous literature searches and studies. Note: Kg product/ha was used for the calculation of exposure in the risk assessment.

Table 9-1 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.	10L/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 * Risk assessment calculated at 8 kg/ha application rate.

IIIM1 9 Fate and behaviour in the environment for the Microbial Pest Control Product (Rationale to waive testing, based on adequacy of information provided for MPCA, to permit an assessment of the fate and behaviour of MPCP in the environment)

Report:	KIIIM1 9/01; [redacted]; [redacted]; [redacted]; [redacted]; 1982; M-416610-01-1
Title:	Fate in model ecosystems of microbial species of potential use in genetic engineering
Report No.:	M-416610-01-1
Document No.:	M-416610-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

Fate and behaviour in soil

Based on available information derived from studies and published literature on *Bacillus subtilis* and *Bacillus amyloliquefaciens* bacteria, the environmental fate and population dynamics the strain QST 713 upon field application of Serenade ASO can be summarized as follows:

Bacillus subtilis and *Bacillus amyloliquefaciens* are a member of the natural micro-flora in soils and occurs without geographical restriction in almost any environmental niche, including the direct human environment. Following an application of Serenade ASO, survival of the endospores of *Bacillus amyloliquefaciens* in soil is very likely for a period of a few months during which time a natural breakdown begins and gradually reduces the numbers of spores remaining. In a dry state endospores can remain viable for several years, vegetative cells, however, are far more rapidly degraded.

It is very unlikely that endospores of *Bacillus subtilis* and *Bacillus amyloliquefaciens* will germinate and grow into vegetative cells, unless encouraging conditions exist, meaning favourable soil pH, soil moisture content, sufficient nutrient availability and lack of competition / predation from other soil micro-organisms. The cells will produce endospores when organic matter, e.g. manure, declines. The survival of *B. subtilis* in soil is a dynamic process consisting of several discernible phases: germination, outgrowth, multiplication, and sporulation in specific habitats, and is influenced by changing conditions regarding soil type, native micro-flora, nutrient availability, and fertilization.

Due to its ubiquitous distribution in soil and the absence of growth, *B. subtilis* and *B. amyloliquefaciens* cells and spores introduced into soils are not expected to exceed the natural level permanently.

Mobility in soil

An evaluation of the probable spread of *B. subtilis* and *B. amyloliquefaciens* in the soil or to associated environments, such as groundwater, is of minor concern, because dispersal of *B. subtilis* and *B. amyloliquefaciens* would lack any hazardous effects. It is generally accepted that population densities decline with increasing soil depth indicating that the level of translocation to deeper layers is negligible. *Bacillus subtilis* and *B. amyloliquefaciens* endospores are reported to as having longevity in groundwater. However, *B. subtilis* is not regarded as an autochthonous inhabitant of aquatic environments and does not find optimal conditions for growth, e.g. waters are poor in organic C. Therefore, proliferation in ground water is not likely to occur. Considering the negligible amount of *B. subtilis* or *B. amyloliquefaciens* spores probably reaching groundwater habitats and the absence of active growth it is thus concluded that no threat of contamination of groundwater exists following applications of Serenade ASO according to GAP.

Possible contamination with metabolite

Persistence of *B. subtilis* and *B. amyloliquefaciens* in soil is restricted to viable spores which are metabolically inactive. Thus, production of new metabolites upon reaching the soil environment can be excluded. Moreover, *B. amyloliquefaciens* QST 713 does not produce metabolites of toxicological concern and no such substances are contained in the end-use product. Therefore, contamination with metabolites is not of relevance for the evaluation of Serenade ASO.

Please refer to the baseline dossier for the background information on fate and behaviour in soil. The calculation was based on the accumulated field rate of Serenade ASO in grapes, with a maximum of 9 applications.

Predicted environmental concentration in soil (PEC_{Soil})

In order to perform a risk assessment for non-target organisms, the actual concentration of Serenade ASO upon nine applications in grapes is calculated as here the highest exposure is expected according to the intended uses. The calculation bases on a maximum application rate of 8 kg Serenade ASO/ha, assuming as a worst case that no degradation occurs between applications. No interception is considered for the calculation. For the risk assessment the resultant load of Serenade ASO will be related to the top 5 cm of soil to achieve the highest theoretical soil concentration.

Summary of the PEC_{Soil} calculations

Critical use	Grapes, maximum of nine applications with 8 Kg Serenade ASO/ha each
Accumulated application rate	72 kg Serenade ASO/ha, 1.008 kg *B. amyloliquefaciens* QST 713/ha, 7.2×10^{13} CFU/ha
Soil density	1.5 g/cm³ (= 75 kg soil/ m²)
Incorporation depth	5 cm layer (= 50 L soil/m²)
Plant interception	Not considered
PEC_{Soil}	96 mg Serenade ASO/kg dry weight soil, 1.34 mg *B. amyloliquefaciens* QST 713/kg dry weight soil, 9.6×10^{7} CFU/kg dry weight soil

Fate and behaviour in water

Aquatic organisms may be exposed to Serenade ASO through spray drift from the application site into adjacent water bodies. The present PEC calculation was performed on the basis of nine applications in grapes, as here the highest exposure of aquatic non-target organisms is to be expected. The maximum drift rate considering 9 applications in vineyards is 6.26% of the applied amount at a distance of 3 m to surface waters. As a worst case, no degradation between the applications is assumed. Drift was calculated according to JKI spray drift data (status from 21.09. 2015).

Summary of the PEC_{SW} calculations

Calculation of the predicted environmental concentration of Serenade ASO in lentic water bodies (PEC_{SW})

Application rate kg/ha	Rate mg/m²	Distance (m)	Drift (%)[b]	Amount of drift		Initial PEC_{SW} [µg/L]	
				g/ha	mg/m²	1 m	30 cm
72[a]	7200	3	6.26	4507.2	450.7	450.7	1502.4[c]

[a] Accumulated application rate of Serenade ASO for GAP directed use in grapes 9 × 8 kg/ha)
[b] According to Julius Kühn Institut[1], status September 2015.
[c] Equivalent to 1.5×10^{6} CFU/L or 21.03 µg *B. amyloliquefaciens* QST 713/L

[1] Basic Drift Values according to Julius Kühn Institut: status September 2015, http://www.jki.bund.de/no_cache/en/startseite/institute/anwendungstechnik/abdrift-eckwerte.html

Due to the PEC_{SW} calculation, the initial concentration of Serenade ASO in 30 cm depth in surface waters is 1502.4 µg/L (21.03 µg *B. amyloliquefaciens* /L) corresponding to 1.5×10^6 CFU/L.

Fate and behaviour in air

Endospores are suitable for aerial distribution as they are easily blown about by wind (please refer to the baseline dossier, Annex II, Doc IIM, Section 5, Point IIM 7.1.3). Therefore, under conditions of use drift spacious transport may occur. Multiplication of *B. amyloliquefaciens* QST 713 in the air, aerosols or clouds can be excluded due to lack of organic matter supply and lack of mineral matrix to adhere to.

Furthermore, unlike chemical products, evaporation and volatility of bacteria is not expected to be a factor to consider in assessing the fate in air. Hence, volatilisation from plant surfaces and from soil can be excluded. An investigation of photochemical-oxidative degradation in air is of no relevance in view of the volatility characteristics of the bacteria. In addition, during distribution of vegetative cells of *B. amyloliquefaciens* QST 713 in air they are exposed to several environmental stress factors (desiccation, UV-radiation, temperature). Therefore, survival of vegetative cells in air is limited and therefore not of relevance for the evaluation of Serenade ASO (please refer to the baseline dossier, Annex II, Doc IIM, Section 5, Point IIM 7.1.3).