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 6

Point IIIM1 10: Effects of the Microbial Pest Control Product on non-target organisms

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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 formulation Serenade ASO was not the representative formulation in the dossier for Annex I inclusion of *B. subtilis* QST 713, here the data of above mentioned product is summarized, since it represents latest information on the *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) for the new representative formulation. For studies previously submitted on the zonal level the reference study citation for these endpoints will be in blue to indicate they have been submitted on the zonal level. All summaries will reflect the name of the study test substance as indicated in the study. New calculated endpoints use the new strain designation of *Bacillus amyloliquefaciens* QST 713.

Critical Good Agricultural Practices for Serenade ASO are summarized in table 10-1. 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. Ecotoxicity data relevant to the MCPA represent the relevant information to be used in the risk assessment of Serenade ASO, as explained below in section IIIM1 10. They are thus used as reference for the risk assessment of Serenade ASO.

Table 10-1 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

*Please note for the purposes of calculating PEC values and risk assement the rate in Kg product/ha and CFU/g values were used as noted in the tables.

Table 10-2 Summary of the PEC 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/ m^2)
Incorporation depth	5 cm layer (= 50 L soil/m^2)
Plant interception	Not considered
Distance	3 m
Drift	2.7%
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
Initial PEC_{SW} (30 cm)	1502.4 µg Serenade ASO/L, 21.03 µg *B. amyloliquefaciens* QST 713/L, 1.5×10^9 CFU/L

***Product applied on L/ha basis, risk calculated in Kg/ha**

IIIM1 10 Rationale to waive additional testing, based on adequacy of information provided for MPCA, to permit an assessment of the impact of the MPCP on non-target organisms

No experimental data on putative effects to non-target species are available for the formulation Serenade ASO, since European data requirement refer to the Technical grade Active Ingredient (TGAI). This information is presented in the baseline dossier for the MCPA *Bacillus amyloliquefaciens* QST 713 (previously designated as *Bacillus subtilis* QST 713). Ecotoxicity data relevant to the MCPA this represent the relevant information to be used in the risk assessment of Serenade ASO (MPCP).

IIIM1 10.1 Effects on birds

No new studies are submitted, assessing the effect of Serenade ASO on birds. Risk assessment, basing on data previously evaluated on the zonal or EU level are summarized below.

Overview and summary

Table 10.1.1 EU Endpoints: Toxicity of *Bacillus subtilis* QST 713, now designated *B. amyloliquefaciens* QST 713, to birds

Study	Test substance	Test species	EU agreed endpoints (SANCO/10184/2003 - rev. final - 14/07/2006)	Endpoints used in risk assessment
Short-term dietary toxicity	QST 713 Technical Powder	Northern bobwhite quail (*Colinus virginianus*)	LD_{50} > 5000 mg QST 713 Technical Powder/kg bw/d (380 mg pure *B. subtilis* QST 713/kg bw/d) (>10^{11} CFU/kg bw/d)	LD_{50} > 380 mg *B. subtilis* QST 713/kg b.w.

Effects on birds for Serenade ASO were not evaluated as part of the EU review of *Bacills subtilis* QST 713 now designated *Bacillus amyloliquefaceins* QST 713. However, further data for Serenade ASO are not relevant as active substance data on toxicity to birds are used and the ingredients in the formulation do not pose a risk to birds. Therefore, all relevant data were assessed in the EU review.

Risk assessments for Serenade ASO with the proposed use pattern are provided here and are considered adequate.

Risk Assessment

Toxicity

The short-term toxicity of QST 713 Technical Powder to *Colinus virginianus* was evaluated (refer to Annex II, Doc IIM, Section 6, Point IIM 8.1). The test substance was administered at a daily dose of 5000 mg/kg bw/day for five days. No treatment related mortalities or effects of QST 713 Technical Powder occurred in the test organism. The acute LD_{50} can be determined to lie above the tested concentration of 5000 mg/kg bw/day.

Table 10.1-2 Summary of avian toxicity endpoint for *Bacillus subtilis* QST 713, now designated *B. amyloliquefaciens* QST 713

Study type	Test substance	Species	Endpoint	Reference
Short-term dietary toxicity	QST 713 Technical Powder	*Colinus virginianus* (Northern bobwhite)	LD_{50} > 5000 mg/kg b.w.[a]	[redacted] et al., 1998 M-473475-01-3

a) Corresponding to > 380 mg pure *B. amyloliquefaciens* QST 713/kg bw/d or > 10^{11} CFU/kg bw/d.

Exposure

Birds are typically exposed to dry residues on their food items following the dilution and spraying of the formulated product. During these processes, much of the formulation constituents are likely to be lost by volatilisation. Therefore, where oral exposure is the main route of exposure, toxicity data for the active substance are used in preference to data from tests with the formulated material. Exposure to Serenade ASO via dermal and inhalation routes is considered unlikely, since at the time of application and for a short period thereafter, most wild mammals will leave the immediate vicinity of spray operations in response to the human disturbance.

The potential exposure of birds to Serenade ASO was estimated following GAP directed applications of the product in the different uses at maximum application rates. The risk assessment for effects on birds is carried out according to the latest draft of the 'European Food Safety Authority Guidance Document on Risk Assessment for Birds and Mammals (EFSA Journal 2009)[1].

Toxicity exposure ratios

Acute toxicity exposure ratio (TER_A)

Birds may be exposed to Serenade ASO as a result of feeding on contaminated vegetation, seeds or insects. Standard exposure scenarios for the intended uses are described in the EFSA Journal[2]. The risk for indicator species of each scenario was assessed in a screening assessment. Data on short-term toxicity are used as they cover acute toxicity to birds.

According to the EFSA Journal (2009)[2] the daily dietary dose (DDD) was calculated for the active substance with the following formulae:

DDD (multiple) = application rate (kg/ha) × shortcut value × MAF

With:

Shortcut value = default parameter combining food intake rate, body weight, concentration of the substance in the diet (based on the 90th percentile residues) and the fraction of diet obtained in the treated area for the bird indicator species/crop combination in question. In case of multiple applications a corresponding MAF (multiple application factor) is considered. The TER value was calculated by dividing the acute endpoint by the daily dietary dose (DDD) for each application rate.

The screening assessment was performed for strawberries and grapes. The screening assessment is shown in **Table 10.1-3**.

[1] European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12): 1438. [139 pp.].

Table 10.1-3 Screening assessment for birds following GAP directed application of Serenade ASO.

Indicator species	Crop	Test item	Toxicity LD_{50}	Application rate[a)]	Max. number of applications	MAF[b)]	Short cut value[c)]	TER (10)
Small omnivorous bird	Strawberries	QST 713 Technical Powder	> 380 mg *B. subtilis* QST 713/kg b.w.	0.112 kg a.s./ha	6	2.2	158.8	> 9.1
	Grapes				9	2.4	95.3	14.8

a) Refers to *B. amyloliquefaciens* QST 713 (corresponding to 8 kg Serenade ASO/ha for the intended uses)
b) MAF according to 6 or 9 successive applications at intervals of 5 days provided in EFSA Guidance document 2009[2]
c) Short cut value based on the 90th percentile of residues provided in EFSA Guidance document 2009[2]

The TER_A values for grapes exceed the Annex VI trigger value of 10, indicating that Serenade ASO poses no risk to birds following application according to the proposed use patterns of these crop scenarios.

Because the resulting acute TER values for strawberries was slightly below the trigger value of 10 a First Tier risk assessment was performed. For the refinement generic focal species and corresponding short cut values depending on the crop stage are provided in the Annex I of the EFSA Guidance document (2009)[2]. According to the intended GAP for Serenade ASO (please refer to Appendix 2 of this section) the risk assessment was performed assuming six applications in strawberries. The risk assessment is shown in **Table 10.1-4** and **Table 10.1-5.**

Table 10.1-4 First Tier risk assessment for birds following application of Serenade ASO in strawberries.

Scenario	Generic focal species	Toxicity LD_{50}	Application rate[c)]	Short cut value[a)]	MAF[b)]	TER (10)
BBCH ≥ 20	Small insectivorous bird "wagtail"	380 mg *B. subtilis* QST 713/kg bw	0.112 kg a.i./ha	25.2	2.2	> 61.2
BBCH ≥ 40	Small omnivorous bird "lark"			9.6		> 160.6
Late applications BBCH 61 - 89	Frugivorous bird "Starling", 100% berries			27.0		> 57.1

a) Short cut value based on the 90th percentile of residues provided in EFSA Guidance document 2009[2].
b) MAF according to six applications at a minimum interval of 5 days provided in EFSA Guidance document 2009[2].
c) Refers to *B. amyloliquefaciens* QST 713 (corresponding to 8 kg Serenade ASO/ha for the intended uses)

The TER_A value, resulting from the First Tier risk assessment, is much higher than the Annex VI trigger value of 10, indicating that Serenade ASO does not pose acute risk to birds following application in strawberries according to the proposed use patterns.

Risk mitigation

[2] European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12): 1438. [139 pp.].

No risk mitigation measures are required.

Short-term and long-term toxicity exposure ratio ($TER_{ST/LT}$)
As the acute TER value indicates no risk to birds and no adverse effects were observed in short-term toxicity studies, no long-term effects are to be expected upon field application of Serenade ASO according to GAP.

Effects on Terrestrial Vertebrates Other Than Birds

In this section, studies are submitted assessing the effect of Serenade ASO on rats. Please refer to Annex III, Doc IIIM1, Points IIIM1 7.1.1 and IIIM1 7.1.3. A summary of the risk assessment in consideration of the current uses and the previously evaluated studies is presented below. Additionally, data from the assessment with a similar formulated product, Serenade AS, containing the same the active ingredient, as well as one study performed with active substance *B. subtilis* QST 713, are presented.
Beside this, no relevant literature was identified to inform the risk assessment of Serenade ASO to terrestrial vertebrates other than birds.

Overview and summary

Table 10.1-5 Ecotoxicological endpoints of *Bacillus subtilis* QST 713, now designated *B. amyloliquefaciens* QST 713 for mammals

Test substance	Test species	EU agreed endpoints (SANCO/10184/2003 – rev. final – 14/07/2006)	Endpoints used in risk assessment	Reference
QST 713 Technical Powder	Rat	$LD_{50} > 1.13 \times 10^8$ CFU/animal (> 5.65×10^8 CFU/kg b.w.[a])	$LD_{50} > 2.5 \times 10^{10}$ CFU/kg bw	[redacted], 1998 (please refer to Annex II, Doc IIM, Point IIM 5.1.1.1 of the EU dossier) M-474035-01-1
QST 713 WP	Rat	$LD_{50} > 5000$ mg/ kg bw (> 2.5×10^{10} CFU/kg b.w.)		[redacted][b], 1998 (please refer to Annex III, Doc IIIM1, Point IIIM1 7.1.1 of the EU dossier) M-474041-01-1
Serenade ASO	Rat	$LD_{50} > 5000$ mg/kg b.w. (> 7×10^{10} CFU/kg b.w.)	$LD_{50} > 7 \times 10^{10}$ CFU/kg b.w. (corresponding to > 5000 mg/kg b.w.)	[redacted], 2015a, please refer to Doc IIIM1, Section 3, Point IIIM1 7.1.1 of this dossier M-527086-01-1
Serenade ASO	Rat	$LC_{50} > 5.91$ mg/L (> 8.27×10^7 CFU/L)		[redacted] 2015b, please refer to Doc IIIM1, Section 3, Point IIIM1 7.1.3 of this dossier M-527088-02-1

a) Assuming a mean body weight of 200 g/animal
b) in the Monograph E.M. [redacted] is mentioned as author of the study although the study director was [redacted]

Effects on mammals for Serenade ASO were not evaluated as part of the EU review of *B. subtilis* QST 713 now designated *B. amyloliquefaciens* QST 713. However, further data on Serenade ASO are not relevant as data on the active substance *B. amyloliquefaciens* QST 713 and a similar formulated product (Serenade AS) on toxicity are used and are considered adequate. Serenade AS, also formulated as a suspension concentrate, contains the same amount of active ingredient as Serenade ASO. Assuming that if even, adverse effects may only be due to consumption of bacterial spores and not due to the co-formulants contained in Serenade ASO, the maximum LD_{50} value referring to the content of *B. amyloliquefaciens* QST 713 is 7×10^{10} CFU/kg bw, corresponding to 5,000 mg Serenade ASO/kg bw. Risk assessments for Serenade ASO basing on the maximum LD_{50} value and the proposed use pattern are provided here and are considered adequate.

Exposure

Mammals are typically exposed to dry residues on their food items following the dilution and spraying of the formulated product. During these processes, much of the formulation constituents are likely to be lost by volatilisation. Therefore, oral uptake by feeding on contaminated food is the main route of exposure. Dermal and inhalation routes of exposure to Serenade ASO are considered unlikely, since at the time of application and for a short period thereafter, most wild mammals will leave the immediate vicinity of spray operations in response to human disturbance. For tomatoes, pepper and aubergine application is solely intended in greenhouses (Please refer to Appendix 2 of this document). Accordingly, mammals will not be exposed to Serenade ASO in this crop scenario. Thus, application in tomatoes, pepper and aubergine was not considered in the risk assessment.

The potential exposure of mammals to Serenades ASO was estimated following GAP directed applications of the product in the different uses at maximum application rates. The risk assessment for effects on mammals is carried out according to the latest draft of the 'European Food Safety Authority Guidance Document on Risk Assessment for Birds and Mammals' (EFSA Journal 2009)[2].

Toxicity exposure ratios

Acute toxicity exposure ratio (TER_A)

Mammals may be exposed to Serenade ASO as a result of feeding on contaminated vegetation, seeds or insects. Standard exposure scenarios for the intended uses are described in the EFSA Journal[2]. The risk for indicator species of each scenario was assessed in a screening assessment. Data on short-term toxicity are used as they cover acute toxicity to mammals.

According to the (EFSA Journal 2009)[2] the daily dietary dose (DDD) was calculated for the active substance with the following formulae:

DDD (multiple) = application rate (kg/ha) × shortcut value × MAF

With:

Shortcut value = default parameter combining food intake rate, body weight, concentration of the substance in the diet (based on the 90th percentile residues) and the fraction of diet obtained in the treated area for the mammal indicator species/crop combination in question. In case of multiple applications a corresponding MAF (multiple application factor) is considered. The TER value was calculated by dividing the acute endpoint by the daily dietary dose (DDD) for each application rate. The screening assessment was performed for use in strawberries and grapes. Details are shown in **Table 10.1-6**.

[2] European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12): 1438. [139 pp.].

Table 10.1-6 Screening assessment for mammals following application of Serenade ASO.

Indicator species	Crop	Test item	Toxicity LD_{50}	Application rate	MAF[a)]	Short cut value[b)]	TER (10)
Small herbivorous mammals	Strawberries	Serenade ASO	$> 7 \times 10^{10}$ CFU/kg bw corresponding to 5000 mg Serenade ASO/kg bw	8 kg Serenade ASO/ha	2.2	136.4	2.1
	Grapes				2.4		1.9

a) MAF according to 6 successive applications in strawberries and 9 successive applications in grapes, provided in EFSA Guidance document 2009[2]

b) Short cut value based on the 90th percentile of residues provided in EFSA Guidance document 2009[2]

The TER_A value for strawberries is below the Annex VI trigger of 10, indicating that mammals might be at risk if Serenade ASO is used in these crops according to GAP. For both grapes and strawberries a First Tier risk assessment was performed. For the refinement, generic focal species and corresponding short cut values depending on the crop stage are provided in the Annex I of the EFSA Guidance document (2009)[2]. The risk assessment is shown in **Table 10.1-7** and **Table 10.1-8**.

Table 10.1-7 First Tier risk assessment for mammals following application of Serenade ASO in grapes.

Scenario	Generic focal species	Toxicity LD_{50}	Application rate	Short cut value[a)]	MAF[b)]	TER (10)
Application crops directed, BBCH ≥ 40	Small herbivorous mammal "vole"	$> 7 \times 10^{10}$ CFU/kg bw corresponding to 5000 mg Serenade ASO/kg bw	8 kg Serenade ASO/ha	40.9	2.4	> 6.4
	Small omnivorous mammal "mouse"			5.2		> 50.1
BBCH < 40	Large herbivorous mammal "lagomorph"			8.1		> 32.2

a) Short cut value based on the 90th percentile of residues provided in EFSA Guidance document 2009[2].

b) MAF according to nine applications at a minimum interval of 5 days provided in EFSA Guidance document 2009[2].

From the obtained TER_A values, small omnivorous ("mouse") and large herbivorous ("lagomorph") mammals exceeded the Annex IV trigger value of 10, while small herbivorous mammals "vole" were below the trigger value. It can be concluded that small herbivorous mammals might be at risk upon GAP directed use of Serenade ASO in grapes. However, no toxicity on mammals was detected when rats were exposed to 5000 mg/kg b.w. during acute oral toxicity test (please refer to Annex III, Doc IIIM1, Point IIIM1 7.1.1). When exposing rats to Serenade ASO at acute inhalation toxicity studies, no mortality or gross abnormalities were detected (please refer to Annex III, Doc IIIM1, Point IIIM1 7.1.3). It was therefore concluded, that Serenade was not classificated as toxic or harmful. Since none of the studies showed toxic effects, and TER values were exceeded the calculated value, no toxicity on mammals is expected upon GAP directed use of Serenade ASO in grapes.

Table 10.1-8 First Tier risk assessment for mammals following application of Serenade ASO in strawberries.

Scenario	Generic focal species	Toxicity LD_{50}	Application rate	Short cut value[a)]	MAF[b)]	TER (10)
BBCH ≥ 40	Large herbivourus mammal "lagomorph"	> 7×10^{10} CFU/kg b.w. corresponding to 5000 mg Serenade ASO/kg bw	8 kg Serenade ASO/ha	14.0	2.4	> 20.3
	Small herbivourous mammal "vole"			54.6		5.2
	Small omnivourous mammal "mouse"			6.9		41.2
BBCH ≥ 20	Small insectivorous mammal "shrew"			5.4		52.6

From the obtained TER_A values, small omnivorous ("vole"), small insectiborous ("shrew") and large herbivorous ("lagomorph") mammals exceeded the Annex IV trigger value of 10, while small herbivorous mammals "vole" were below the trigger value. It can be concluded that small herbivorous mammals might be at risk upon GAP directed use of Serenade ASO in strawberries. However, no toxicity on mammals was detected when rats were exposed to 5000 mg/kg b.w. during acute oral toxicity test (please refer to Annex III, Doc IIIM1, Point IIIM1.7.1.1). When exposing rats to Serenade ASO at acute inhalation toxicity studies, no mortality or gross abnormalities were detected. It was therefore concluded, that Serenade was not classificated as toxic or harmful. Since non of the studies showed toxic effects, and TER values were greater as calculated, no toxicity on mammals is expected upon GAP directed use of Serenade ASO in strawberries.

Moreover, basing on previous evaluated studies it was concluded that *B. subtilis* QST 713, does not pose risk on mammals. Calculation to assess the risk upon GAP directed use in strawberries and grapes is presented below:

According to the (EFSA Journal 2009), the daily dietary dose (DDD) was calculated for the active substance with the following formulae:

DDD (multiple) = application rate (kg/ha) × shortcut value × MAF

With:

Shortcut value = default parameter combining food intake rate, body weight, concentration of the substance in the diet (based on the 90th percentile residues) and the fraction of diet obtained in the treated area for the mammal indicator species/crop combination in question. In case of multiple applications a corresponding MAF (multiple application factor) is considered. The TER value was calculated by dividing the acute endpoint by the daily dietary dose (DDD) for each application rate. The screening assessment was performed for use in strawberries and grapes. Details are shown in **Table 10.1-9**.

Table 10.1-9 Screening assessment for mammals following application of Serenade ASO.

Indicator species	Crop	Test item	Toxicity LD_{50}	Application rate	MAF[a)]	Short cut value[b)]	TER (10)
Small herbivorous mammals	Strawberries	Serenade ASO	$> 7 \times 10^{10}$ CFU/kg bw corresponding to 5000 mg Serenade ASO/kg bw	8 kg Serenade ASO/ha	2.2	118.4	11.5
	Grapes				2.4		9.1

a) MAF according to 6 successive applications in strawberries and 9 successive applications in grapes, provided in EFSA Guidance document 2009[2]

b) Short cut value based on the 90th percentile of residues provided in EFSA Guidance document 2009[2]

The TER_A value for strawberries is the Annex VI trigger of 10, indicating that mammals are not at risk if Serenade ASO is used in these crops according to GAP. For grapes a First Tier risk assessment was performed. For the refinement, generic focal species and corresponding short cut values depending on the crop stage are provided in the Annex I of the EFSA Guidance document (2009)[2]. The risk assessment is shown in **Table 10.1-10**.

Table 10.1-10 First Tier risk assessment for mammals following application of Serenade ASO in grapes.

Scenario	Generic focal species	Toxicity LD_{50}	Application rate	Short cut value[a)]	MAF[b)]	TER (10)
Application crops directed, BBCH ≥ 40	Small herbivorous mammal "vole"	$> 7 \times 10^{10}$ CFU/kg bw corresponding to 5000 mg Serenade ASO/kg bw	8 kg Serenade ASO/ha	40.9	2.4	> 30.5
	Small omnivorous mammal "mouse"			5.2		> 239.6
BBCH ≥ 40	Large herbivorous mammal "lagomorph"			8.1		> 153.8

c) Short cut value based on the 90th percentile of residues provided in EFSA Guidance document 2009[2].

d) MAF according to nine applications at a minimum interval of 5 days provided in EFSA Guidance document 2009[2].

From the obtained TER_A values, all exceeding the Annex IV trigger value of 10 it can be concluded that mammals are not at risk upon GAP directed use of Serenade ASO in grapes.

Risk mitigation

No risk mitigation measures are required.

Short-term and long-term toxicity exposure ratio ($TER_{ST/LT}$)

Short-term effects on mammals for Serenade ASO were not evaluated as part of the EU review of *B. subtilis* QST 713, now designated *B. amyloliquefaciens* QST 713. However, further data on Serenade ASO are not relevant as data from another Serenade fungicide containing *B. subtilis* QST 713, now designated *B. amyloliquefaciens*, are available and have been already assessed in the EU review.

Table 10.1-11 Short term inhalation toxicity to mammals

Substance	Species	Endpoint	Value (CFU *B. subtilis* QST 713 /animal)	Report
Serenade Biofungicide (WP formulation)*	Rat	LD_{50}	> 5×10^8 CFU/animal (3350 g/animal	[redacted], 2004 (this report was submitted in December 2004 and is cited in Addendum 2 to the Monograph, date of issue: 30.11.2005) M-474026-01-1

During the 28-day inhalation study conducted with a Serenade fungicide containing *B. subtilis* QST 713 ([redacted], 2004; please refer to Annex II, Doc IIM, Point IIM 5.1.4 in the EU dossier of *B. subtilis* QST 713, there were no signs of toxicity observed and the microorganism proofed to be non-pathogenic as spores were cleared from all body organs within eight weeks after the last day of exposure. Although inhalation is not the main exposure route of plant protection products to wild animals thriving in agricultural fields, on the basis of the 28-day study and from the absence of toxicity in the three presented acute oral toxicity studies it can be concluded that no long term effects on mammals are to be expected upon field application of Serenade ASO.

IIIM1 10.2 Effects on aquatic organisms

No new studies are submitted assessing the effect of formulated product Serenade ASO on aquatic organisms. Instead data from the assessment with *Bacillus subtilis* QST 713 Technical product are presented for all aquatic non-target species. Please note, that *B. amyloliquefaciens* QST 713 was previously designated as *B. subtlis* QST 713. No relevant literature was found to inform the risk assessment of Serenade ASO to aquatic organisms.

Table 10.2-1 EU Endpoints: Toxicity of *Bacillus subtilis* QST 713 (now designated as *B. amyloliquefaciens* QST 713) for aquatic organisms

Test item	Test species	EU agreed endpoints (SANCO/10184/2003 - rev. final – 14/07/2006)	Endpoints used in risk assessment	Reference
Fish				
QST 713 Technical Powder	*Oncorhynchus mykiss*	30-day (semi-static) LC_{50} = 3.24×10^9 CFU/L NOEC = 1.72×10^9 CFU/L	NOEC = 1.72×10^9 CFU/L	[redacted], 1998a (please refer to Annex II Doc IIM, Section 6, Point IIM 8.2.1 of the EU dossier) M-473642-02-1
		30-day (semi-static) LC_{50} = 1.4×10^{10} CFU/L NOEC = 1.7×10^9 CFU/L		[redacted] et al., 2001a (submitted in June 2002, cited in Addendum 1 to the Monograph, date of issue: 04.12.2002) M-473492-01-1

Test item	Test species	EU agreed endpoints (SANCO/10184/2003 - rev. final – 14/07/2006)	Endpoints used in risk assessment	Reference
Aquatic invertebrates				
QST 713 Technical Powder	*Daphnia magna*	48-hour (static) $EC_{50} = 2.16 \times 10^9$ CFU/L NOEC = 2.6×10^8 CFU/L	Acute toxicity: $EC_{50} = 2.16 \times 10^9$ CFU/L	[redacted] 1998b (please refer to Annex II Doc IIM, Section 6, Point IIM 8.2.2.1 of the EU dossier) M-473465-01-2
	Daphnia magna	21-day (semi-static) $EC_{50} = 1.6 \times 10^9$ CFU/L NOEC = 7.9×10^8 CFU/L		[redacted] et al., 2001b (submitted in June 2002; cited in Addendum 1 to the Monograph, date of issue: 04.12.2002) M-473458-02-1
	Daphnia magna	21-day (semi-static) $EC_{50} = 6 \times 10^8$ CFU/L NOEC = 1.5×10^8 CFU/L	Long term toxicity: NOEC = 1.5×10^8 CFU/L	[redacted] & [redacted], 1998c (please refer to Annex II Doc IIM, Section 6, Point IIM 8.2.2.2 of the EU dossier) M-473638-02-1
	Palaemonetes pugio	30-day (semi-static) NOEC = 2.7×10^9 CFU/L		[redacted], 2001 (This report was submitted in June 2002 and is cited in Addendum 1 to the Monograph, date of issue: 04.12.2002) M-473476-01-1
Single cell algae				
QST 713 Technical Powder	*Scenedesmus subspicatus*	72-hour NOEC = 3.3×10^8 CFU/L	NOEC > 3.3×10^8 CFU/L	[redacted], 2000 (please refer to Annex II Doc IIM, Section 6, Point IIM 8.2.3 of the EU dossier) M-473469-01-1

Further data on Serenade ASO are not relevant as active substance data on toxicity are used and the ingredients in the formulation do not pose a risk to aquatic species. Therefore, all relevant data were already assessed in the EU review. Risk assessments for Serenade ASO with the proposed use pattern are provided here and are considered adequate.

Fish
Two semi-static renewal tests on rainbow trout (*Oncorhynchus mykiss*) were conducted with QST 713 Technical Powder over a period of 30 days ([redacted] & [redacted], 1998a; [redacted] et al., 2001a). Exposure through aqueous and dietary routes caused no adverse effects to *O. mykiss* based on parameters of survival, infectivity and/or pathogenicity. The lowest LC_{50} value was estimated to be 3.24×10^9 CFU/L and the no-observed-effect-concentration (NOEC) was determined to be = 1.72×10^9 CFU/L.

Aquatic invertebrates
One short term (48-hour) and two long-term (21-day) studies on the effect of QST 713 Technical Powder on daphnids (*Daphnia magna*) were conducted ([redacted] & [redacted] 1998b and c; [redacted] et al, 2001b). As a conclusion, an EC_{50} value of 2.16×10^9 CFU *B. subtilis*/L was regarded as threshold value for short-term toxicity of *B. subtilis* QST 713 to daphnids (*Daphnia magna*). The lowest no-observed-effect-concentration (NOEC) in the 48-hour assay was determined to be 2.6×10^8 CFU *B. subtilis* QST 713/L. The lowest EC_{50} value resulting from the 21-day assays was determined to be 6×10^8 CFU/L and the lowest NOEC was 1.5×10^8 CFU/L.
One long-term (30-day) toxicity study on the effect of QST 713 Technical Powder on the Grass shrimp (*Palaemonetes pugio*) was carried out ([redacted], 2001). The lowest no-observed-effect-concentration (NOEC) for this species was determined to be 2.7×10^9 CFU *B. subtilis* QST 713/L.

Algae
An acute toxicity study (72-hour) on *Scenedesmus subspicatus* was conducted with QST 713 Technical Powder ([redacted], 2000). No adverse effects of QST 713 Technical Powder were observed at any test concentration. Therefore, no EC values could be calculated. The lowest no-observed-effect-concentration (NOEC) for this species was determined to be NOEC = 3.3×10^8 CFU *B. subtilis* QST 713/L.

Exposure
Aquatic organisms may be exposed to Serenade ASO and *Bacillus amyloliquefaciens* QST 713 through spray drift. Exposure of aquatic organisms from this route was estimated by calculating Predicted Environmental Concentration in surface water (PEC_{SW}) (see Annex III, Doc IIIM1, Section 5).
For 9 applications in vineyards (drift value of 6.26%) assuming worst case conditions of no degradation of *B. amyloliquefaciens* QST 713 between the spraying resulting in an accumulated application rate of 72 kg Serenade ASO/ha, the initial concentration of Serenade ASO and *B. amyloliquefaciens* QST 713 in 30 cm depth in surface waters is as follows:

Table 10.2-2 PEC_{SW} values for Serenades ASO

Test substance	PEC_{SW}
Serenade ASO	1502.4 µg/L
B. amyloliquefaciens QST 713	21.03 µg/L
B. amyloliquefaciens QST 713	1.5×10^6 CFU/L

Toxicity exposure ratios
The initial risk assessments were carried out by comparing the PEC_{SW} values with the acute and long-term toxicity endpoints. The toxicity/exposure ratio (TER) for aquatic organisms is derived from the EC_{50} or LC_{50} value (acute risk assessment) or the NOEC (chronic risk assessment) according to the formula:

$$TER = \frac{EC_{50,}\ LC_{50} \text{ or NOEC [CFU/L]}}{PEC_{SW} \text{ [CFU/L]}}$$

TER_A for fish
Due to the absence of toxicity in the semi static studies conducted over a period of 30 days no acute risk for fish is expected upon short term exposure to Serenade ASO**.**

TER_{LT} for fish

The lowest long-term toxicity endpoint for rainbow trout was used as it covers the acute risk assessment. The resulting long-term TER value for *B. amyloliquefaciens* QST 713, based on the maximum PEC_{SW} value following nine applications in vineyards × 3 m from the application site is shown below.

Table 10.2-3 Fish long-term TER value for Serenade ASO (*Bacillus subtilis* QST 713, now designated *B. amyloliquefaciens* QST 713)

Test organism	Test substance	NOEC	PEC_{SW}	TER_{LT}	Trigger value
Oncorhynchus mykiss	QST 713 Technical Powder	1.72×10^9 CFU/L	1.5×10^6 CFU/L	1147	10

The TER value for *B. amyloliquefaciens* QST 713 is above the Annex VI trigger value of 10, indicating that GAP directed use of Serenade ASO poses no risk to fish.

TER_A for *Daphnia*

The acute Serenade ASO TER for *Daphnia magna* was calculated using the calculated PEC_{SW} at 3 m from the application site following based on the maximum PEC_{SW} value following nine applications in vineyards x 3 m from the application site. The resulting TER_A is presented below:

Table 10.2-4 Acute TER value for *D. magna* for Serenade ASO

Test organism	Test substance	48 hour EC_{50}	PEC_{SW}	TER_A	Trigger value
Daphnia magna	QST 713 Technical Powder	2.16×10^9 CFU/L	1.5×10^6 CFU/L	1440	100

The acute TER value for *B. amyloliquefaciens* QST 713 is above the Annex VI trigger value of 100, indicating that GAP directed use of Serenade ASO poses no risk to fish.

Risk mitigation

No risk mitigation measures are required.

TER_{LT} for *Daphnia*

The TER_{LT} of Serenade ASO for *Daphnia magna* was calculated using the calculated PEC_{SW} at 3 m from the application site and the lowest NOEC obtained in the 21-day semi-static tests with *B. amyloliquefaciens QST 713*. The resulting TER_{LT} is presented below:

Table 10.2-5 *Daphnia* TER_{LT} value for Serenade ASO

Test organism	Test substance	NOEC	PEC_{SW}	TER_{LT}	Trigger value
Daphnia magna	QST 713 Technical Powder	1.5×10^8 CFU/L	1.5×10^6 CFU/L	100	10

From the TER_{LT} value exceeding the trigger of 10 no long-term risk for daphnids is indicated. Prolonged exposure, however, is not likely to occur due to the restricted persistence of *B. amyloliquefaciens* in water.

TER_{LT} for algae

The acute risk for algae from exposure to Serenade ASO was assessed using the NOEC value for B. subtilis QST 713 Technical Powder, which was determined to be 3.3×10^8 CFU/L. The resulting TER, calculated on the basis of the PEC_{SW} value for nine applications in vineyards at 3 m from the application site, is given in the **Table 10.2-6**.

Table 10.2-6 Algae TER_{LT} value for Serenade ASO

Test organism	Test substance	72 hours NOEC	PEC_{SW}	TER_A	Trigger value
Scenedesmus subspicatus	QST 713 Technical Powder	3.3×10^8 CFU/L	1.5×10^6 CFU/L	220	10

The TER value is above the Annex VI trigger value of 10, indicating that application of Serenade ASO according to the proposed label uses poses no risk to algae.

Risk mitigation

No risk mitigation measures are required.

IIIM1 10.3 Effects on bees

No new studies are submitted assessing the effect of formulated product Serenade ASO on bees.

No relevant literature was found to inform the risk assessment of Serenade ASO to bees.

Higher tier field studies for evaluation of contact and oral toxicity of Serenade WP, containing the same active ingredient, *Bacillus subtilis* QST 713, now designated *B. amyloliquefaciens* QST 713, have been evaluated as part of the EU review of *Bacillus subtilis* QST 713 . Based on these studies, calculations of a hazard quotient (HQ) for risk assessment were not considered to be necessary as no adverse effects were noted under field conditions of use.

Effects on bumblebees (*Bombus terrestris*) were reported in a study conducted by [redacted] et al. (2009), which is submitted and described in detail in Annex II, Doc IIIM, Point IIM 8.7. In this study, adult workers were exposed to Serenade by contact application or administration of treated pollen or treated sugar water. After 11 weeks, contact application and orally application via treated sugar water led to mortality of bumblebees. Unfortunately, no results on mortality were reported for the previous weeks, although examination was performed weekly. Moreover, data of the negative control is not presented clearly, however it is stated that it should be zero. Nevertheless, possible toxicity of *B. subtilis* QST 713 on bumble bee was clearly presented, although methods were not performed under good laboratory practice (GLP). Moreover, it has to be considered that agricultural use of *B. subtilis* QST 713 does not imply a continuous administration over such a long time as carried out by [redacted] et al. (2009). The authors themselves stated that the assessment did not reflect the realistic conditions in field. However, when administered in a pollen patty or as a dry formulation the authors reported no adverse effects indicating that the toxicity was due to exposure to wet product for a long term continuous exposure and not likely under more realistic exposure conditions.

However, no effects were observed in a study conducted by [redacted] (2006), submitted in Annex II; Doc IIM, Point IIM 8.7. Hereby, bumblebees (*Bombus terrestris*) were exposed to Serenade MAX, containing *B. subtilis* QST 713. Direct and indirect exposure of 300 g Serenade MAX/hL on bees did not show effects on bees. Mortality was almost zero percent in all treated greenhouses throughout the entire study period. Also foraging activity was high in treated greenhouses in comparison to the untreated control. Moreover, activity at the hives was not influenced by direct or indirect treatment. Thus, Serenade MAX, containing the active substance *B. subtilis* QST 713 was evaluated to be safe for bumblebees.

Table 10.3-1 Ecotoxicological endpoints for honey bees

Test item	EU agreed endpoints (SANCO/10184/2003 - rev. final – 14/07/2006)	Reference
QST 713 Technical Powder	5-day dietary: LC_{50} ~ 8,900 ppm Equivalent to ~ 1.8×10^8 CFU/mL diet	[redacted] et al., 1998a (please refer to Annex II, Doc IIM, Section 6 Point IIM 8.3 of the EU dossier) M-473639-01-2
Serenade WP	30-day field study No effects at application rates of 1.12 kg *Bacillus subtilis* QST 713/ha	[redacted], 2000 (please refer to Addendum 1 to the Monograph, date of issue: 04.12.2002) M-473494-01-1
QST 713 Technical Powder	30-day dietary effect study (larvae) predicted LD_{50} between 4,700,000 and 6,714 mg/kg bw	[redacted] & [redacted], 2003 (please refer Addendum 2 to the Monograph, date of issue: 30.09.2005) M-473477-01-1
QST 713 Technical Powder	30-day laboratory study NOEL 10,000 to 100,000 ppm	[redacted], 2004 (please refer Addendum 2 to the Monograph, date of issue: 30.09.2005) M-486885-01-1

Effects on bees for Serenade ASO were not evaluated as part of the EU review of *Bacillus subtilis* QST 713, now designated *Bacillus amyloliquefaciens*. However further data on Serenade ASO are not relevant as active substance data on toxicity are used and the ingredients in the formulation do not pose a risk to bees. Hence, all relevant data were already assessed in the EU review. A comparison of field application rates of Serenade ASO/ *B. subtilis* QST 713 and the dosages which were tested in the available studies is provided here instead of a complete risk assessment and is considered adequate.

Risk Assessment

A study assessing the dietary toxicity and pathogeneity of *B. subtilis* on the honey bee, *Apis mellifera*, was conducted ([redacted] et al., 1998a). The 5-day LC_{50} value was determined to be 1.8×10^8 CFU/mL diet. According to the GAP directed use of Serenade ASO concentration of *B. amyloliquefaciens* QST 713 in the tank mix suspension is calculated for application in grapes, as here the highest concentration is expected: as per hectare 8L Serenade ASO, (useing 8 kg for risk calculation) corresponding to 8×10^{12} CFU are used, suspended in a water volume of 500-1000 L water. Assuming 500 L as worst case, the concentration of *B.amyloliquefaciens* in the tank mix will be 1.6×10^{10} CFU/L or 1.6×10^7 CFU/mL. Hence, the LC_{50} value is 11 times higher than the maximum concentration of *B.amyloliquefaciens* in the tank mix suspension, indicating that application of Serenade ASO does not pose risk to honey bees.

A field study with Serenade Biofungicide Wettable Powder and free-living honey bees was conducted in a 30-day period ([redacted], 2000). No adverse effects of Serenade WP were observed at an application rate 6 × 1.12 kg *B. subtilis* QST 713/ha with a 5-day interval. In comparison, the maximum single application rate of Serenade ASO is 8 kg/ha, corresponding to 0.112 kg *B.amyloliquefaciens* QST 713/ha. Assuming a worst case of nine applications, and considering no degradation of bacteria on leaf and fruit surfaces and on flowers, the amount of *B. amyloliquefaciens* QST 713 would result in 1.008 kg/ha (72 kg/ha Serenade ASO). The highest accumulated application rate of Serenade ASO (1.008 kg *B. amyloliquefaciens* QST 713/ha) is far below the amount of Serenade Biofungicide Wettable Powder (6.72 kg *B. subtilis* QST 713/ha) that was used in the field study. Therefore, it is appropriate to conclude that also the use of Serenade ASO, which

contains no formulants of ecotoxicological concern compared to Serenade Biofungicide Wettable Powder, at the highest application rate in grapes, is not expected to be hazardous to honeybees.

The comparison of the endpoints resulting from the above studies to the maximum application rates of Serenade ASO is summarised in **Table 10.3-2**.

Table 10.3-2 Honey bee toxicity endpoints for Serenade ASO

Test substance	Endpoint	Tested value	Application rate of Serenade ASO/ *B. amyloliquefaciens* QST 713
QST 713 Technical Powder	LC_{50}	1.8×10^8 CFU/mL diet	4.17×10^7 CFU/mL tank mix suspension
Serenade WP	NOEL	6.72 kg *B. subtilis* QST 713 /ha	1.008 kg *B. amyloliquefaciens* /ha

From the results of all studies it can be concluded, that application of Serenade ASO according to Good Agricultural Practice intended uses, does not pose a risk to honey bees.

Risk mitigation

No risk mitigation measures are required.

IIIM1 10.4 Effects on arthropods other than bees

No new studies are submitted assessing the effect of formulated product Serenade ASO on arthropods other than bees. No relevant literature was found to inform the risk assessment of Serenade ASO to arthropods other than bees.

Previously submitted studies, evaluated for the risk assessment of Serenade ASO on arthropods other than bees, are presented below. Instead data from the assessment with Technical product (Powder) and Serenade WP, a similar formulated product containing the same active ingredient as Serenade ASO (Please refer to Annex III, Doc I-IIIM1), were asessed.

Table 10.4-1 Ecotoxicological endpoints for arthropods other than bees

Test item	EU agreed endpoints (SANCO/10184/2003 - rev. final – 14/07/2006)	Test species	Endpoints used in risk assessment [a)]	Reference
QST 713 Technical Powder	LR_{50} > 16 kg QST 713 TP/ha >1.22 kg *B. subtilis* QST 713 /ha	*Typhlodromus pyri*	*Aphidius rhopalosiphi* *Typhlodromtus pyri* LR_{50} : > 16 kg QST 713 Technical Powder/ha 1.22 kg *B. subtilis* QST 713 /ha	[redacted], 2000 (please refer to Annex II, Doc IIM, section 6, point IIM 8.4) M-473491-01-2
QST 713 Technical Powder	LR_{50} > 16 kg QST 713 TP/ha >1.22 kg *B. subtilis* QST 713 /ha	*Aphidius rhopalosiphi*		[redacted], 2000 (please refer to Annex II, Doc IIM, section 6, point IIM 8.4) M-473472-01-2
QST 713 Technical Powder	NOEC = 60,000 ppm = 4.56 g/L diet	*Hippodamia convergens*		[redacted] et al, 1998b (please refer to Annex II, Doc IIM, section 6, point IIM 8.4) M-473489-01-2
QST 713 Technical Powder	NOEC = 600 ppm = 0.046 g/L diet	*Chrysopeda carnea* larvae		[redacted] et al, 1998c (please refer to Annex II, Doc IIM, section 6, point IIM 8.4) M-473488-01-2
QST 713 Technical Powder	NOEC = 1,730 ppm = 0.33 g/L diet	*Nasonia vitripennis*		[redacted] et al., 2001 (This report was submitted in June 2002 and is cited in Addendum 1 to the Monograph, date of issue: 04.12.2002) M-473490-01-1
QST 713 Technical Powder	NOEC = 6,000 ppm = 0.46 g/L diet	*Nasonia vitripennis*		[redacted] et al, 1998d (please refer to Annex II, Doc IIM, section 6, point IIM 8.4) M-473640-01-2
Serenade WP	Accumulated test rate: 42.3 kg/ha Serenade WP = 4.26 kg *B. subtilis* QST 713 /ha	*Typhlodromus pyri*		[redacted], 2000a (submitted in June 2002, cited in Addendum 1 to the Monograph, date of issue: 04.12.2002) M-486909-01-1
Serenade WP	Accumulated test rate: 43.0 kg/ha Serenade WP = 4.33 kg *B. subtilis* QST 713 /ha	*Typhlodromus pyri*		[redacted], 2000b (submitted in June 2002, cited in Addendum 1 to the Monograph, date of issue: 04.12.2002) M-486910-01-1

[a)] Risk has been assessed for those studies where results have been expressed in terms of the amount of test substance/ha.

Effects of the formulation Serenade ASO on arthropods were not evaluated as part of the EU review of *Bacillus subtilis* QST 713, now designated as *B. amyloliquefaciens* QST 713. However, additional data on Serenade ASO are considered not necessary since data of a similar formulated

product and active substance data are available and the ingredients in the formulation Serenade ASO do not pose a risk to arthropods, Hence all relevant data have been already assessed during the EU review. Risk assessments for Serenade ASO with the proposed use pattern are provided in the following and are considered adequate.

Toxicity

The acute toxicity and effect of *B. subtilis* QST 713 on reproduction of the predatory mite *Typhlodromus pyri* and the aphid parasitoid *Aphidius rhopalosiphi* (Hymenoptera, Braconidae) were determined in laboratory glass plate tests. Only slight effects on mortality (12.5 and 13%, respectively) were observed at the tested rate of 16 kg QST 713 TP/ha (corresponding to 1.22 kg *B. subtilis* QST 713/ha). Due to the absence of mortalities exceeding 50%, the LR_{50} can be predicted to exceed 1.22 kg *B. subtilis*/ha.

The risk assessment strategy used here follows the approach recommended in the ESCORT 2 *guidance document* (2000)[3] and the *EC Guidance Document on Terrestrial Ecotoxicology*[4].

An overview on the obtained data is provided in **Table 10.4-2**. The data can be applied for the risk assessment of Serenade ASO as formulants do not pose a risk to arthropods.

Table 10.4-2 Serenade ASO - Toxicity to non-target arthropods

Test substance	Species	Exposed life stage	Study type	LR_{50} (kg product/ha)	Reference
QST 713 Technical Powder	*Aphidius rhopalosiphi*	Adult	Acute laboratory (glass plate)	>16 kg QST 713 TP >1.22 kg *B. subtilis* QST 713	[redacted], 2000
	Typhlodromus pyri	Proto-nymphs		>16 kg QST 713 TP >1.22 kg *B. subtilis* QST 713	[redacted], 2000

Exposure

In-field

Non-target arthropods living in the crop can be exposed to residues from Serenade ASO by direct contact either as a result of overspray or through contact with residues on plants and soil or in food items. The maximum number of successive treatments for Serenade ASO in grapes is 9 and the maximum application rate is 8 kg/ha. The in-field exposure is calculated according to ESCORT 2 (2000 [3]) using the following equation:

$$PER_{In\text{-}field} = \text{Maximum application rate [g a.s/ha]} \times MAF$$

Due to nine applications, a default MAF value (leaf) of 3.5 was adopted.

Table 10.4-3 In-field PER values for application of Serenade ASO

Compound referred to	Application rate	MAF	PER (foliar)
B. amyloliquefaciens QST 713	122 g a.s./ha[a)]	3.5	427 g a.s./ha

a) Corresponding to 8 kg Serenade ASO/ha

[3] Escort 2 (2000): Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods. From the ESCORT 2 workshop

[4] EC Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC, SANCO/10329, 17 October 2002.

Off-field

Risk assessment of areas immediately surrounding the crop is considered important since these areas represent a natural reservoir for immigration, emigration and reproduction of arthropod populations and provide increased species diversity. Exposure of non-target arthropods living in off-field areas to Serenade ASO will mainly be due to spray drift. Off-field areas are assumed to be densely vegetated and thus spray drift is unlikely to reach bare ground. Therefore, evaluation of exposure *via* soil residues in off-field areas was not considered. The off-field exposure value was calculated from in-field exposure in conjunction with drift values published by the JKI (2006)[5] as shown in the following equation:

$$PER_{Off\text{-}field} = \frac{\text{Maximum in-field exposure} \times (\%\ \text{drift}/100)}{\text{Vegetation distribution factor}}$$

Vegetation distribution factor: The model used to estimate spray drift was developed for drift onto a two-dimensional water surface and, as such, does not account for interception and dilution by three-dimensional vegetation in off-crop areas. Therefore, a vegetation distribution or dilution factor is incorporated into the equation when calculating off-field exposure, to be used in conjunction with toxicity endpoints derived from two-dimensional (glass plate or leaf disc) studies. A dilution factor of 10 is recommended by ESCORT 2 guidance document (2000)[4].

The $PER_{off\text{-}field}$ was calculated for the use of Serenade ASO in grapes because here the highest exposure is expected due to the highest drift rate and number of applications. For nine applications the drift value at 3 m distance is 6.26 % of the application rate (90th percentile drift). The drift factor (% drift/100) is therefore 6.26/100 = 0.0626.

The resulting $PER_{off\text{-}field}$ value is shown in **Table 10.4-4**.

Table 10.4-4 Off-field foliar Predicted Environmental Rates (PER) for Serenade ASO

Study type	Maximum in-field foliar PER [a] (g a.s./ha)	Drift factor (% drift/100)[a]	Vegetation distribution factor	Off-field foliar PER (g a.s./ha)
Glass plate	427	0.0626	10	2.67

[a] For grapes, 9 applications

Risk assessment

The risk to non-target arthropods is assessed using the approach recommended in the published ESCORT 2[3] guidance document (2000) and the *EC Guidance Document on Terrestrial Ecotoxicology*[4].

In-field

The potential risk of Serenade ASO to in-field non-target arthropods was assessed by calculation of the hazard quotient (HQ = exposure/toxicity) with the predicted environmental rate (PER) and the lowest lethal rate (LR_{50}) values according to the following formula:

$$\text{In field } HQ = \frac{\text{In - field PER}}{LR_{50}}$$

The HQ trigger for Tier I laboratory is 2. The resulting $HQ_{in\text{-}field}$ values are presented in **Table 10.4-5**.

[5] Julius Kuhn Institute spray drift data from 27. March 2006, http://www.jki.bund.de/fileadmin/dam_uploads/_AT/abdrift-eckwerte/Abdrifteckwerte_xls.xls

[3] Escort 2 (2000): Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods. From the ESCORT 2 workshop

[4] EC Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC, SANCO/10329, 17 October 2002.

Table 10.4-5 In-field HQs for non-target arthropods

Species	LR_{50} (g a.s./ha)	In-field foliar		Trigger value
		PER (g a.s./ha)	HQ	
Typhlodromus pyri	1220	427	< 0.35	2
Aphidius rhopalosiphi				

Conclusion: the in-field HQ values indicate that GAP directed application of Serenade ASO poses no risk to in-field non-target arthropods.

Off-field

In order to assess the potential risk of Serenade ASO to off-field non-target arthropods, the predicted environmental rate (**Table 10.4-4**) is compared with the toxicity endpoints according to the following formula:

$$\text{Off - field HQ} = \frac{\text{PER}_{\text{off-field}}\ (\text{g a.s./ha})}{\text{LR}_{50}\ (\text{g a.s./ha})} \times \text{Correction factor}$$

The HQ trigger for off-field scenarios is 2.

Correction factor: ESCORT 2 guidance document (2000)[3] recommends including an uncertainty (safety) factor of 10 into the off-field HQ calculation to account for uncertainty with the extrapolation from *T. pyri* and *A. rhopalosiphi* as indicator species, to all off-field non-target arthropods. $HQ_{off-field}$ values are given in **Table 10.4-6.**

$HQ_{off-field}$ values are given in **Table 10.4-6.**

Table 10.4-6 Off-field HQ values for non-target arthropods

Species	LR_{50} (g a.s./ha)	Off-field foliar PER(g a.s./ha)	Correction factor	Off-field foliar HQ	*Trigger value*
Typhlodromus pyri	1220	2.67	10	< 0.022	*2*
Aphidius rhopalosiphi					

The off-field HQ value for the tested non-target arthropods falls below the trigger value of 2, indicating that GAP directed application of Serenade ASO does not pose an unacceptable risk to non-target arthropods in off-field areas.

As HQ values for the in-crop scenarios as well as for the off-crop scenarios are below the trigger of 2, the ESCORT 2 document and SANCO/10329/2002[3] demand no further higher tier testing.

Risk mitigation measurements

Not required.

IIIM1 10.5 Effects on earthworms

No new studies are submitted assessing the effect of formulated product Serenade ASO on earthworms. Please note that *B. amyloliquefaciens* QST 713 was previously designated as *B. subtilis* QST 713. Summary of assessment data is presented below. No relevant literature was found to inform the risk assessment of Serenade ASO to earthworms.

[3] Escort 2 (2000): Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods. From the ESCORT 2 workshop

Instead data of a laboratory study determining the acute toxicity of Serenade WP (content of *B. subtilis* QST 713 of 5.07×10^9 CFU/g or 14.92% dry weight) to the earthworm *Eisenia foetida* is presented. This study has already been evaluated as part of the EU review of *B. subtilis* QST 713.

Table 10.5-1 Ecotoxicological endpoints for earthworms

Test item	EU agreed endpoints (SANCO/10184/2003 - rev. final – 14/07/2006)	Endpoints used in risk assessment
Serenade WP[a)]	LC_{50} 14 d > 1000 mg/kg artificial soil or > 149.7 mg *B. subtilis* QST 713/ kg dry weight soil	LC_{50} > 149.7 mg *B. subtilis* QST 713/ kg dry weight soil

a) Content of *B. subtilis* QST 713 in Serenade WP: 5.07×10^9 CFU/g or 14.92% dry weight

Risk assessments for Serenade ASO with the proposed use pattern are provided here and are considered adequate.

Toxicity

The acute toxicity of the formulation Serenade WP to the earthworm *Eisenia foetida* was determined in a laboratory study ([redacted], 2002, cited in Addendum 1 to the Monograph, date of issue: 04.12.2002). The median lethal concentration LC_{50} of the test item to *Eisenia foetida* determined after 14 days exposure was shown to be greater than 1000 mg Serenade WP/kg artificial soil or149.7 mg *B. subtilis*/ kg dry weight soil.

Earthworm toxicity endpoints are summarised in **Table 10.5-2**.

Table 10.5-2 Acute earthworm toxicity endpoint for Serenade ASO

Test substance	Endpoint	Value	Reference
Serenade WP[a)]	LC_{50}	149.7 mg *B. subtilis* QST 713/ kg dry weight soil	[redacted], 2002 M-473495-01-1

a) Content of *B. subtilis* QST 713 in Serenade WP: 5.07×10^9 CFU/g or 14.92% dry weight

Exposure

The exposure to earthworms was estimated by calculating the maximum predicted environmental concentrations in soil (PEC_{Soil}) (please refer to Annex III, Doc IIIM1, Section 5, Point IIIM1 9). The PEC_{Soil} value was calculated for 9 applications of 8 kg Serenade ASO/ha assuming no degradation of the product and the active ingredient occurs between the treatments and no plant interception as a worst case. Under these conditions and based on standard assumptions for the soil density and the incorporation depth, the PEC_{Soil} was determined to be 96 mg Serenade ASO/kg dry weight soil (1.34 mg *B. amyloliquefaciens* QST 713/kg dry weight soil). In terms of CFU, this is equivalent to 9.6×10^7 CFU/kg dry weight soil.

Toxicity exposure ratios: TER_A and TER_{LT}

Acute risk

The potential acute risk of Serenade ASO to earthworms was assessed by comparing the maximum instantaneous PEC_S with the 14-day LC_{50} value to generate the acute TER value. The TER_A was calculated as follows:

$$TER_A = \frac{LC_{50}\ (mg/kg)}{PEC_S\ (mg/kg)}$$

The resulting TER_A value is shown in **Table 10.5-3**.

Table 10.5-3 Acute TER value for earthworms

Compound referred to	LC_{50}	Maximum PEC_S for Serenade ASO	TER_A	Limit
Serenade ASO	> 149.7 mg *B. subtilis*/ kg dry weight soil	1.34 mg *amyloliquefaciens* QST 713/kg dry weight soil	> 111.7	10

The acute TER value is much higher than the Annex VI acute trigger value of 10, indicating that GAP directed application of Serenade ASO poses no acute risk to earthworms.

Long-term risk
Due to the absence of acute toxicity no adverse effects on earthworms are to be expected even upon prolonged exposure to Serenade ASO or *B. amyloliquefaciens* QST 713.

Risk mitigation
No risk mitigation measures are regarded necessary.

IIIM1 10.6 Effects on soil micro-organisms

No new studies are submitted assessing the effect of formulated product Serenade ASO on soil micro-organisms. No relevant literature was found to inform the risk assessment of Serenade ASO to soil micro-organisms.

It is referred to the literature submitted for the active substance *B. amyloliquefaciens* QST 713. The ingredients of the preparation Serenade ASO, formulated as a suspension concentrate, are inert, non toxic and impose no environmental or health risk (confidential data, please refer to Doc J-IIIM1). Therefore, information on the microbial pest control agent, *B. amyloliquefaciens* QST 713 and information on closely related *B. subtilis* QST 713 (as it was previously designated) is considered applicable and relevant with regard to the evaluation of the formulated product.
Studies on the effects on micro-organisms were not considered to be necessary due to the following generally accepted aspects in the ecology and environmental behaviour of *B. amyloliquefaciens* and *B. subtilis* derived from open literature:
The active substance *B. amyloliquefaciens* is a member of the natural micro-flora in soils and occurs without geographical restriction in almost any environmental niche, including the immediate human environment. It is an autochthonous soil micro-organism and has originally been isolated from soil in a peach orchard in the U.S.A. Therefore, its possible multiplication in this natural habitat does not disturb the natural micro-flora. Although it is commonly found in soil, it occurs in almost any environment, including niches in kitchens and bathrooms. For more information, please refer to Annex II, Doc IIM, Point IIM 8.10.

According to the Working Document to the Environmental Safety Evaluation of Microbial Biocontrol Agents (SANCO/12117/2012-rev. 0, September 2012)[3]. Tests assessing possible effects of microbial pesticides on soil micro-organisms are not stringently significant for the following reasons:

- Risk caused by introduction of microorganisms to the soil microbial community is minimal, because soil microflora naturally fluctuates in time and space. The natural populations are well adapted to their habitat and exhibit many defence mechanisms in order to assure their survival.
- Soil microbial communities show good resilience, and populations are able to recover even upon extreme decimation e.g. by methyl bromide.

Additionally, a literature search was conducted to identify latest studies regarding possible effects of *B. amyloliquefaciens* on soil microorganisms. Both studies confimed the above mentioned statements, since only minor influence of *Bacillus* application on soil microorganisms were observed. For more information, please refer to Annex II, Doc IIM, Point IIM 8.10.

[3] Working Document to the Environmental Safety Evaluation of Microbial Biocontrol Agents, SANCO/12117/2012-rev.0, September 2012, EUROPEAN COMMISSION HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL Directorate E – Safety of the food chain Unit E.3 – Chemicals, contaminants, pesticides.

In conclusion, negative effect to the soil microflora following application of Serenade ASO according to GAP directed uses are not expected.

Risk mitigation
No risk mitigation measures are regarded necessary.

IIIM1 10.7 Additional studies

No additional studies are required.