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 III, Section 3

Point IIIM1 7: Toxicological Studies and Exposure Data and Information for the Microbial Pest Control Product

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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[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 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). The for information submitted only on the zonal level when different from the new information will appear in blue font.

Critical Good Agricultural Practices for Serenade ASO are summarized in the table below.

Table 7-1 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 7 Toxicological Studies and Exposure Data and Information for the Microbial Pest Control Product

IIIM1 7.1 Acute toxicity studies

Overall summary

The acute toxicity, irritancy and sensitisation potential of the suspension concentrates Serenade ASO or Serenade AS containing *B. subtilis* QST 713 as active substance have been studied in rats, rabbits or Guinea pigs. Serenade ASO contains the same certified limits of active ingredient as Serenade AS. All of the inert ingredients in Serenade ASO are from the United States Environmental Protection Agency´s Inert List, 4(a) or 4(b). Under the United States Department of Agriculture's National Organic Program the active ingredient also qualifies as an organic material labelled in the USA and some European Member State for use in organic agriculture.

For details of the formulation Serenade ASO and a comparison of the additives of Serenade ASO and Serenade AS formulations, please refer to Doc IIII Point IIIM1 1.7.2.1. The results of toxicological studies conducted with the plant protection products Serenade ASO containing *B. subtilis* QST 713 as active ingredient are summarised in **Table 7.1-1.**

Table 7.1-1: Summary of acute toxicity studies used for the evaluation of Serenade ASO

Study type	Test item	Dose level	Findings	Report
Acute oral toxicity rat	Serenade ASO	5000 mg/kg bw Corresponding to 5.5 x 10 ^{10} CFU/kg	LD_{50} > 5000 mg/kg bw	Refer to Point IIIM1 7.1.1 [redacted] (2015a)
Acute dermal toxicity rat	Serenade ASO	5000 mg/kg bw corresponding to at least 2 × 10^9 CFU /kg bw	LD_{50} > 2000 mg/kg bw	Refer to Point III M 7.1.2 [redacted] (2008)
Acute inhalation toxicity rat	Serenade ASO	5.19 mg/L Corresponding to 7 x 10^7 CFU/L	No adverse effects LC_{50} > 5.19 mg/L	Refer to Point IIIM1 7.1.3 [redacted] (2015b)
Dermal irritation rabbit	Serenade ASO	500 mg/animal corresponding to at least 0.5 × 10^9 CFU /animal	Non-irritating	Refer to Point IIIM1 7.1.4 [redacted] (2008a)
Eye irritation/ corrosion rabbit	Serenade ASO	0.1 mL/animal	Non-irritating	Refer to Point IIIM1 7.1.5 [redacted] (2008b)
Skin sensitation guinea pig	Serenade AS*	100% Serenade AS	Non-sensitising	Refer to Point IIIM1 7.1.6 [redacted] (2001)

*non-oraganic formulation no longer produced. Same CFU count as Serenade ASO

The microbial plant protection product Serenade ASO containing *B. subtilis* strain QST 713 as active substance has a low potential of toxicity following oral, dermal or inhalation exposure.

The suspension concentrate plant protection product Serenade ASO was not irritating to the skin or eyes of the rabbit.

Serenade ASO containing *B. subtilis* strain QST 713 as active substance was not a sensitiser in the modified Buehler method in Guinea pigs. However, in accordance with the PRAPeR Expert Meeting on micro-organisms in June 2009 the following labelling is proposed:

"*Bacillus subtilis* QST 713 may have the potential to provoke sensitizing reactions."

IIIM1 7.1.1 Acute oral toxicity

Report: KIIIM1 7.1.1/01; [redacted]; 2015; M-527086-01-1
Title: Serenade ASO: Acute oral toxicity - Up-and-down procedure in rats
Report No.: 40862
Document No.: M-527086-01-1
Guideline(s): U.S. EPA Health Effects Test Guidelines, OCSPP 870.1100 (2002)
OECD Guidelines for the Testing of Chemicals, Test No. 425 (2008)
Guideline deviation(s): not specified
GLP/GEP: **yes**

Material and methods: Test substance identified as Serenade ASO with lot number 102000027846, LJSB000447. Composition: *Bacillus subtilis* strain QST 713 - 1.34%, other ingredients (nominal) - 98.66%. Brown suspension. Expiration date: March 5, 2017.
Animals: Three female nulliparous and non-pregnant Sprague-Dawley derived albino rats of 9 – 10 weeks and 151 – 166 g, received from SAGE® Labs.
The animals were singly housed in plastic solid bottom polycarbonate caging. Enrichment (e.g., toy) was placed in each cage. Litter paper was placed beneath the cage and was changed at least three times per week. The ranges of animal room temperature and relative humidity were 20 – 23°C and 40 – 58%, respectively. Air changes consisted in 12 per h with a photoperiod of 12 h light/dark cycle. Food and water, consisting on Harlan Teklad Global 16% Protein Rodent Diet® #2016 and tap water, respectively, were provided *ad libitum*, with the exception of food during fasting. Animals were acclimatised during 7 – 12 days prior dosing.

Prior to each dosing, experimentally naive rats were fasted overnight by removing the feed from their cages. During the fasting period, the rats were examined for health and weighed (initial). Three healthy, female rats (not previously tested) were selected for test.

The test substance was administered as received to the stomach using a stainless steel ball-tipped gavage needle attached to an appropriate syringe. Following administration, each animal was returned to its designated cage. Feed was replaced approximately 3-4 hours after dosing. The animals were observed for mortality, signs of gross toxicity, and behavioral changes approximately 30 minutes post-dosing, during the first several hours post-dosing and at least once daily thereafter for 14 days after dosing. Observations included gross evaluation of skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern. Particular attention was directed to observation of tremors, convulsions, salivation, diarrhea, and coma. Individual body weights of the animals were recorded prior to test substance administration (initial) and again on Days 7 and 14 (terminal) following dosing. All rats were euthanized via CO_2 inhalation at the end of the 14-day observation period. Gross necropsies were performed on all animals. Tissues and organs of the thoracic and abdominal cavities were examined.

Findings: All animals survived test substance administration, gained body weight, and appeared active and healthy during the study. There were no signs of gross toxicity, adverse clinical effects, or abnormal behavior. No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period.
Conclusion: Under the conditions of this study, the acute oral LD_{50} of Serenade ASO is greater than 5000 milligrams per kilogram of body weight in female rats.

It is concluded that Serenade ASO does not warrant classification as being toxic or harmful on the basis of its acute oral toxicity.

IIIM1 7.1.2 Acute percutaneous (dermal) toxicity

The following acute dermal toxicity study in rats was performed on Serenade ASO containing *B. subtilis* QST 713 as active substance: The results are summarised in **Table 7.1-1**. A detailed summary is provided in support of the assessment.

Report:	KIIIM1 7.1.2/01; [redacted]; 2008; M-456164-01-1
Title:	Acute dermal toxicity study of Serenade ASO in rats - according to EC method B.3. (92/69/EEC) and OECD guideline 402 - Limit test
Report No.:	21360
Document No.:	M-456164-01-1
Guideline(s):	according to EC method B.3. (92/69/EEC) and OECD guideline 402
Guideline deviation(s):	none
GLP/GEP:	**yes**

Materials and Methods: The study was conducted during the period 11.04.-25.04.2007 by [redacted]. The test material corresponded to Serenade ASO (QST 713 strain of *B. subtilis*); Lot No. T-732; reported titer: ≥1 × 10^9 CFU/g, 1.34% dry weight; analyzed titer: 1.10 × 10^{10} CFU/g.

In an acute dermal toxicity study a single dose (limit test) of 2000 mg/kg bw of the test substance, corresponding to at least 2 × 10^9 CFU of *B. subtilis* QST 713/kg bw, was applied for 24 h to 10 CD/Crl:CD(SD) rats (5 per sex) in a volume of 1.90 mL/kg bw. The test substance was applied to shaved and intact skin (5 × 6 cm, corresponding to approximately 1/10 of body surface). After the exposure period the occlusion was removed. Throughout the 14 day observation period, animals were observed for changes of body weight or behavioural pattern, as well as for irritations/changes of skin and fur at least once a day. At the end of this period, all animals were sacrificed and subjected to gross pathological examination.

Findings: No clinical signs of toxicity were observed, body weight gain was in the expected range. Gross macroscopic inspection did not reveal any substance-related findings. Since no mortalities occurred, no LD_{50} could be calculated.

Conclusions: The acute lethal dermal dose of Serenade ASO was found to be greater than 2000 mg/kg bw corresponding to at least 2 × 10^9 CFU *B. subtilis* QST 713 per kg bw.

It is concluded that Serenade ASO does not require labelling or classification with regard to dermal toxicity.

IIIM1 7.1.3 Acute inhalation toxicity to rats

Report:	KIIIM1 7.1.3/01; [redacted]; 2015; M-527088-02-1
Title:	Serenade ASO: Acute inhalation toxicity in rats - Amended final report
Report No.:	40863
Document No.:	M-527088-02-1
Guideline(s):	U.S. EPA Health Effects Test Guidelines, OCSPP 870.1300 (1998) OECD Guidelines for the Testing of Chemicals, Test No. 403 (2009)
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Material and methods: Test substance identified as Serenade ASO with lot number 102000027846, LJSB000447. Composition: *Bacillus subtilis* strain QST 713 - 1.34%, other ingredients (nominal) - 98.66%. Brown suspension. Expiration date: March 5, 2017.
Animals: Ten Sprague-Dawley derived albino rats (5 per sex) of 9 – 10 weeks and 284 – 334 g (males) and 193 – 201 (females), received from SAGE® Labs.
The animals were housed in plastic solid bottom polycarbonate caging. Enrichment (e.g., toy) was placed in each cage. Litter paper was placed beneath the cage and was changed at least three times per week. The ranges of animal room temperature and relative humidity were 19 – 23°C and 41 – 57%, respectively. Air changes consisted in 12 per h with a photoperiod of 12 h light/dark cycle. Food and water, consisting on Harlan Teklad Global 16% Protein Rodent Diet® #2016 and tap water, respectively, were provided *ad libitum*, except during exposure. Animals were acclimatised during 14 days prior dosing.

The test substance was aerosolized as received and kept on a magnetic stirrer during aerosolization.

Prior to initiation of the full inhalation study, pre-test trials were conducted to establish generation procedures to achieve, to the extent possible, the desired chamber concentration (5.0 mg/L) and desired particle size distribution (mass median aerodynamic diameter between 1 and 4 µm). The procedures and aerosolization equipment used in the full test were based on the results of pre-test trial number 13 which provided a gravimetric chamber concentration of 5.13 mg/L and a mass median aerodynamic diameter of 2.14 µm.

A nose-only inhalation chamber was used for exposure. Animals were individually housed in polycarbonate holding tubes which seal to the chamber with an "O" ring during exposure. The base unit terminates the chamber with a 0.5-inch diameter tube for discharged air. Filtered generator air was supplied to the spray atomization nozzle by an air compressor, and measured with a Mass Flow Controller. Additional filtered mixing air from the same air compressor, measured with a Mass Flow Controller, was introduced into the chamber to help uniformly distribute the test atmosphere by creating a vortex at the chamber inlet. Chamber airflow was monitored throughout the exposure period and recorded periodically. The exposure was conducted under slight negative pressure. The temperature and relative humidity within the exposure chamber as well as the room were monitored continuously during exposure, and were measured with a temperature-humidity monitor. Temperature and relative humidity values were recorded every 15 minutes for the first hour of exposure and approximately every 15 or 30 minutes thereafter. The test atmosphere was generated using a nebulizer. The test substance was metered to the atomization nozzle through Tygon® tubing, using a peristaltic pump. Gravimetric samples were withdrawn at 6 intervals from the breathing zone of the animals. Samples were collected using 37 mm glass fiber filters (Whatman™ GF/B) in a filter holder attached by ¼ inch Tygon® tubing to a vacuum pump. Filter papers were weighed before and after collection to determine the mass collected. This value was divided by the total volume of air sampled to determine the chamber concentration. Sample airflows were measured using a Mass Flow Controller. An eight-stage 1 ACFM Andersen Ambient Particle Sizing Sampler was used to assess the particle size distribution of the test atmosphere. Samples were withdrawn from the breathing zone of the animals at two intervals. The filter paper collection stages were weighed before and after sampling to determine the mass collected upon each stage. The mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were calculated using two-cycle logarithmic probit axes. The animals were exposed to the targeted chamber concentration for at least 4 hours. The exposure period was extended beyond 4 hours to allow the chamber to reach equilibrium (T99). At the end of the exposure period, the generation was terminated and the chamber was operated for at least 15

minutes further with clean air to allow the test atmosphere to fully dissipate. At the end of this period the animals were removed from the exposure tubes. Prior to being returned to their cages, excess test substance was removed from the fur of each animal by rinsing with tap water and wiping with clean paper towels.

On the day of and prior to exposure, the rats were examined for health and weighed. Ten healthy, naive rats (five males and five females; not previously tested) were selected for test. Individual body weights of the animals were recorded prior to test substance exposure (initial) and again on Days 1, 3, 7, and 14 (terminal). All animals were observed for mortality during the exposure period. The animals were examined for signs of gross toxicity, and behavioral changes upon removal from the exposure tube and at least once daily thereafter for 14 days. Observations included gross evaluation of skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern. Particular attention was directed to observation of tremors, convulsions, salivation, diarrhea, and coma. All rats were euthanized via CO_2 inhalation on Day 14. Gross necropsies were performed on all animals. Tissues and organs of the thoracic and abdominal cavities were examined.

Findings: The chamber and nominal chamber concentrations were 5.19 mg/L and 173.53 mg/L, respectively. The average mass median aerodynamic diameter was estimated to be 2.35 µm based on graphic analysis of the particle size distribution as measured with a 1 ACFM Andersen Ambient Particle Sizing Sampler with an average geometric standard deviation of 2.32.
All animals survived exposure to the test atmosphere and gained body weight during the study. Following exposure, all rats exhibited irregular respiration. However, all animals recovered by Day 3 and appeared active and healthy for the remainder of the 14-day observation period. No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period.
Conclusion: Under the conditions of this study, the single exposure acute inhalation LC_{50} of Serenade ASO is greater than 5.19 mg/L in male and female rats.

It is concluded that Serenade ASO does not warrant classification as being toxic or harmful on the basis of its acute inhalation toxicity.

IIIM1 7.1.4 Skin irritation

Report:	KIIIM1 7.1.4/01; [redacted]; 2008; M-456190-01-1
Title:	Acute dermal irritation/corrosion test (patch test) of Serenade ASO in rabbits - according to EC method B.4. (2004/73/EEC) and OECD guideline 404 (Final report dated May 30, 2007)
Report No.:	21361
Document No.:	M-456190-01-1
Guideline(s):	according to EC method B.4. (2004/73/EEC) and OECD guideline 404
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Materials and methods: The study was conducted during the period 03.04.-07.04.2007 by [redacted]. The test material corresponded to Serenade ASO (QST 713 strain of *B. subtilis*); Lot No. T-732; reported titer: ≥1 × 10^9 CFU/g, 1.34% dry weight; analyzed titer: 1.10 × 10^{10} CFU/g.

The test substance was applied to the shaved, intact dorsal skin of three male Himalayan rabbits at a dosage of 500 mg per animal, corresponding to at least 0.5 × 10^9 CFU *B. subtilis* QST 713 per animal (0.5 mL test substance per patch). The test substance was covered with a gauze patch to ensure semi-occlusive conditions and fixed with non-irritating tape for the exposure period. Neighboured untreated skin served as a control. Exposure was terminated after 4 hours by removal of the patches and animals were observed for irritations after 60 minutes, 24, 48, and 72 hours.

Findings: Under the conditions of the test, a single animal (no. 3) showed an erythema (grade 1) 1 hour after removal of the patch. No systemic intolerance reactions were observed throughout the observation period.

Conclusion: Serenade ASO containing *B. subtilis* QST 713 as active ingredient is considered to be non-irritating to skin, accordingly, it does not warrant classification regarding skin irritation.]IIIM1IIIM1

IIIM1 7.1.5 Eye irritation

Report:	KIIIM1 7.1.5/01; [redacted]; 2008; M-456195-01-1
Title:	Acute eye irritation/corrosion test of Serenade ASO in rabbits - according to EC method B.5. (2004/73/EEC) and OECD guideline 405 - (Final report dated May 30, 2007) - Amendment no. 1 go final report
Report No.:	21362
Document No.:	M-456195-01-1
Guideline(s):	according to EC method B.5. (2004/73/EEC) and OECD guideline 405
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Materials and Methods: The study was conducted during the period 05.04. – 08.04.2007 by [redacted]. The test material corresponded to Serenade ASO, Lot No. T-732; reported titer: ≥1 × 10^9 CFU/g, 1.34% dry weight; analyzed titer: 1.10 × 10^{10} CFU/g.

Three male Himalayan rabbits received a single dose of 0.1 mL of Serenade ASO by instillation into the right eye. The test substance was placed into the conjunctival sac and in order to prevent loss of the material, the lids were gently held together for approximately one second. As a control, the left eye remained untreated. Exposure was stopped by washing the eyes with 20 mL aqueous sodium chloride solution 24 hours after instillation. Evaluation of eye irritant reactions (including opacities, conjunctival redness, chemosis and damage of the iris) was performed 1, 24, 48, and 72 hours after treatment.

Findings: Under the conditions of the test, all animals were found to exhibit grade 1 conjunctival redness 1 hour after instillation, in animal no.1 until 48 hours after instillation. No effects to the corneae and irises of the animals as well as systemic intolerance reactions were observed.

Conclusions: Serenade ASO containing *B. subtilis* QST 713 as active substance is considered to be non-irritating to eyes, it does not warrant classification or labelling regarding eye irritation.]IIIM1IIIM1

IIIM1 7.1.6 Skin sensitisation

The following labelling phrase that is required for all micro-organisms according to the PRAPeR Expert Meeting on Microorganisms from June 2009 is proposed: "Contains *Bacillus amyloliquefaciens* QST 713. Micro-organisms may have the potential to provoke sensitising reactions."

In addition, according to the Advisory Committee on Pesticides (ACP) at its 355th meeting, the label of the product should include the following phrase: "Wear suitable protective clothing (coveralls), suitable protective gloves and suitable respiratory protective equipment (disposable filtering facepiece respirator to at least EN149 FFP3 or equivalent) when handling the concentrate or applying the product."

The following study is submitted to support the EU reviewe however the default labelling should apply. The following skin sensitisation study in Guinea pigs was conducted on Serenade AS

containing the same certified limits of *B. subtilis* QST 713 active ingredient as Serenade ASO. Since the additives in Serenade ASO have a more favourable toxicological profile, Serenade ASO should be considered even more toxicologically benign than Serenade AS. The results are summarised in **Table 7.1-1**. A detailed summary is provided in support of the assessment.

Report:	KIIIM1 7.1.6/01; [redacted]; 2001; M-456206-01-1
Title:	Dermal sensitization study (closed-patch repeated insult) in Guinea pigs with Serenade AS
Report No.:	012774-1
Document No.:	M-456206-01-1
Guideline(s):	EPA OPPTS Guideline 870.2600 - JAPAN MAFF Guideline 59 NohSan - OECD Guideline 406
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Materials and methods: The study was conducted during the period 20.09. – 27.10.2000 by [redacted]. The test material corresponded to Serenade AS, lot no. 54.70.1AS; reported titer: $\geq 1 \times 10^9$ CFU/g; 1.34 % dry weight; analyzed titer: 3.1×10^9 CFU/g.

The skin sensitising properties of Serenade AS were evaluated using the modified Buehler method. During the induction phase a concentration of 100% Serenade AS was administered dermally to the backs of Hartley Guinea pigs once a week for three consecutive weeks. Dinitrochlorobenzene (DNCB), 0.2% (w/v) in 80% ethanol, was administerd concurrently as a positive control during the induction phase. Two weeks following the last induction, the animals were challenged. During the challenge phase an irritation control group was used to differentiate between dermal reactions produced by irritation and those produced by sensitization.

During the challenge phase, the animals in the test group received single administrations of Serenade AS at approximately the maximum non-irritating concentration of 100% as recieved by the sponsor. The positive control group received single administrations of DNCB at a concentration of 0.07% (w/v) in acetone. The third group of animals (irritation control group) received at the time of challenge only, single administrations of the same concentrations of test and positive control materials administered to the other two groups.

Findings: The positive control material (DNCB) did elicit dermal sensitisation in the postive control group animals. At challenge Serenade AS produced no dermal responses in the test group annimals and only one incidence of very slight erythema in the irritation control group.

Conclusion: The test substance, Serenade AS, demonstrated no potential to produce dermal sensitisation when administered by the modified Buehler method to Hartley guinea pigs.

It is concluded that Serenade ASO, containing *B. subtilis* QST 713 as active ingredient does not warrant classification and labelling regarding skin sensitisation.

IIIM1 7.2 Operator, bystander and worker exposure: monitoring data

Report:	KIIIM1 7.2/01; [redacted]; 2015; M-532269-01-1
Title:	Statement concerning hazards to man during the use or handling of bacilus subtilis strain QST713
Report No.:	M-532269-01-1
Document No.:	M-532269-01-1
Guideline(s):	not specified
Guideline deviation(s):	not specified
GLP/GEP:	**no**

Report:	KIIIM1 7.2/02; [redacted]; 2015; M-532275-01-1
Title:	Statement concerning hazards to man during the use or handling of bacilus subtilis strain QST713
Report No.:	M-532275-01-1
Document No.:	M-532275-01-1
Guideline(s):	not specified
Guideline deviation(s):	not specified
GLP/GEP:	**no**

[*Bacillus subtilis*] *Bacillus amyloliquefaciens* acts in a highly specific manner and is not pathogenic to mammals. This has been shown in many tests on toxicity, pathogenicity and infectiveness to vertebrates, all without adverse effects.

No toxicological adverse effects have been observed on personnel in research or industrial mass production of the active substance *B. subtilis* QST 713 over a period of more than 20 years ([redacted], 2015; [redacted], 2015). Because inerts ingredients used in the preparation are of negligible toxicity as well, an unacceptable risk for operators, workers or bystanders is not anticipated with the use of Serenade ASO.

Due to the lack of toxicity, infectivity and pathogenicity concerns no reference values need to be established, and an exposure assessment for operators, workers and bystander is not required.

IIIM1 7.3 Operator and bystander exposure: reporting of hypersensitivity incidents before and after registration

No cases on hypersensitivity have been reported in production or application of Serenade ASO.

IIIM1 7.4 Safety data sheet for each additive

Serenade ASO does not contain ingredients in concentrations of toxicologically critical concern. The properties of non-active ingredients and their toxicological data are provided in Annex IIIM1, Document J, Safety Data Sheets for non-active substances.

IIIM1 7.5 Supplementary information on all data points in part 7: Effects on human health if it is recommended that MPCP be tank-mixed with an adjuvant or another pest control product

Serenade ASO may be used in combinations with adjuvants or other pest control products. Due to the nature of this biological plant protection product, no influence on the toxicological profile of *B. amyloliquefaciens* QST 713 is to be anticipated from interactions with adjuvants, chemical or other biological plant protection products.

IIIM1 7.6 Summary and evaluation of health effects

All submitted toxicological studies and supplemental information on (*Bacillus subtilis) B. amyloliquefaciens* QST 713 and the formulated product Serenade ASO prove that these are non-toxic and non-infectious to mammals and pose no health risk for operators, bystanders or workers. However, in accordance with the PRAPeR Expert Meeting on micro-organisms in June 2002 the following labelling is proposed:

"Contains *Bacillus amyloliquefaciens* QST 713. Micro-organisms may have the potential to provoke sensitising reactions."

Table 7.6-1 Summary of acute toxicity studies on Serenade ASO

Study type	Test item	Dose level	Findings	Report
Acute oral toxicity rat	Serenade ASO	5000 mg/kg bw Corresponding to 5.5×10^{10} CFU/kg	No mortality, no clinical signs and no toxicity	KIIIM1 7.1.1/01 [redacted] (2015a)
Acute inhalation toxicity rat	Serenade ASO	5.19 mg/L Corresponding to 7×10^{7} CFU/L	No mortality, no clinical signs and no toxicity	KIIIM1 7.1.3/01 [redacted] (2015b)
Acute dermal toxicity rat	Serenade ASO	2000 mg/kg bw corresponding to at least 2×10^{9} CFU /kg bw	LD50 > 2000 mg/kg bw	KIIIM 7.1.2 [redacted] (2008)
Dermal irritation rabbit	Serenade ASO	500 mg/animal corresponding to at least 0.5×10^{9} CFU/animal	Non-irritating	KIIIM1 7.1.4 [redacted] (2008a)
Eye irritation/ corrosion rabbit	Serenade ASO	0.1 mL/animal	Non-irritating	KIIIM1 7.1.5 [redacted] (2008b)