Monograph

15 May 2001

Bacillus subtilis
strain QST 713

Volume 1

Report and
Proposed Decision

Rapporteur Member State: Germany

Contents

Level 1

Bacillus subtilis strain QST 713

Statement of Subject Matter and Purpose of Monograph

1 Statement of subject matter and purpose for which the monograph was prepared

1.1 Purpose for which the monograph was prepared (Dossier Document A)

This monograph is submitted to support first inclusion of the new active substance *Bacillus subtilis*, strain QST 713 in Annex I of the Council Directive 91/414/EEC.

1.2 Summary and assessment of information relating to collective provision of dossiers (Dossier Document B)

As Agra Quest is the only notifier of the active ingredient Bacillus subtilis QST 713, there is no relevance.

1.3 Identity of the organism (Annex IIB 1)

1.3.1 Name and address of applicant(s) for inclusion of the organism in Annex I (Annex IIB 1.1)

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1.3.2 Manufacturer of *Bacillus subtilis* (QST 713 Technical) (Annex IIB 1.2):

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1.3.3 Name and species description (Annex IIB 1.3)

The species *B. subtilis* is a bacterium with an ellipsoidal endospore which does not extend the mother cell. The first description of the species is from Cohn (1872). Ehrenberg (1835) formerly described this species as *Vibrio subtilis* (Gordon *et al.*, 1973). The genus *Bacillus* (Priest, 1993) belongs to the family Bacillaceae among the group gram-positive eubacteria (Schlegel, 1985).
Using the available morphological, physiological and biochemical data, the strain QST 713 was clearly identified as *Bacillus subtilis*.
This strain originates from a naturally occurring wild type, isolated from soil in California (USA) in 1995.

The strain QST 713 has been added to the internationally accepted Agriculture Research Culture Collection (NRRL), Illinois, USA, code number NRRL B-21661.

1.3.4 Composition of the material used for manufacturing of formulated products (Annex IIB 1.4)

The content of pure micro-organism in QST 713 Technical is 14.6 % by weight on average and in terms of colony forming units 5 x 10^{10} cfu/g are stated. QST 713 Technical is the spray dried end-product of a fermentation process using *B. subtilis* strain QST 713 cultures. It was determined to be 100 % pure for *B. subtilis* and did not contain any other micro-organism.

1.4 Identity of the plant protection product (Annex IIIB 1)

1.4.1 Trade name or proposed trade name and manufacturer`s development code number of the preparation (Annex IIIB 1.3)

Trade Name: Serenade™ WP

Code Number: QRD 133 WP

1.4.2 Applicant (Annex IIIB 1.1)

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1.4.3 Manufacturer of the preparation and the micro-organism (Annex IIIB 1.2)

Manufacturer of active substance: AgraQuest
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1.5 Use of the plant protection product

1.5.1 Field of use (Annex IIB 3.2; Annex IIIB 3.1)

Serenade™ is envisaged to be used in fields and under protected cultivation in several horticultural crops and in viticulture.

1.5.2 Mode of action (Annex IIIB 3.2)

Nature of the effects on harmful organisms:

Fungistatic, fungicidal, bactericidal, indirectly via induction of resistance in the host plant.

Serenade™ WP is acting fungistatic and fungitoxic by disruption of hyphae following contact with the fungal pathogen on the leaf surface. High efficacy of this plant protection product is provided when the *B. subtilis* cells colonise the leaf surface to form a protective layer before fungal attack has occurred. Besides antagonism nutrient competition is involved in the mode of action and more importantly *B. subtilis* induces systemic resistance response of the plant, indicated by enhanced peroxidase production.

1.5.3 Summary of intended uses (Annex IIB 3.3; Annex IIIB 3.3 to 3.7, 3.9)

The intended uses of Serenade™ WP envisaged are in viticulture, pome fruit, stone fruit in the field and lettuce under field conditions and protected cultivation as well. The application rates per treatment for orchards is 5-15 kg/ha, in viticulture and lettuce 4-12 kg/ha. The maximum numbers of applications are 16 in pome fruit against scab, but only 4 against fire blight. In stone fruit 10 and in viticulture 8 numbers of applications are envisaged. In the case of lettuce there are no number of applications given because after planting the fungicide agent should be sprayed in an interval of 5 to 7 days up to the day of harvest. In all cases the application method is spraying at infestation or according to local extension service. In the case of the control of fire blight this time of application is during blossom. For further details see table below.

List of uses supported by available data

Crop and/ or situation (a)	Member State or Country	Product name	F G or I (b)	Pests or Group of pests controlled (c)	Formulation		Application			Product application rate per treatment (g)			PHI (days) (h)	Remarks (i)
					Type (d)	Conc. of as (e)	method kind	growth stage (f)	number min max	kg/100 L	water l/ha min max	kg/ha min max		
Orchards, Apple, Pear	North- and South-Europe	Serenade™ WP	F	*Venturia inaequalis* (scab) *Erwinia amylovora* (fire blight)	WP	5 x 10^9 cfu/g	spraying	BBCH 10 to 72 during blossom	1 - 16 4	1 (i.e. 10^{12} cfu)	500 - 1.500	5 - 15	---	*
Orchards, stone fruits		Serenade™ WP	F	*Monilia*	WP	5 x 10^9 cfu/g	spraying	BBCH 55 to 69 BBCH 70 to 84 BBCH 85 to 89	4 4 2	1 (i.e. 10^{12} cfu)	500 - 1.500	5 - 15	---	* **
Grapevines	Middle- and South-Europe	Serenade™ WP	F	*Uncinula necator (Oidium)* *Botrytis cinerea*	WP	5 x 10^9 cfu/g	spraying	BBCH 55 to 75 BBCH 68 to 81	1 - 8 1 - 4	1 (i.e. 10^{12} cfu)	400 - 1.200	4 - 12	---	*
Lettuce	North- and South-Europe	Serenade™ WP	F, G	*Bremia lactucae*	WP	5 x 10^9 cfu/g	spraying	after planting	--	1 (i.e. 10^{12} cfu)	400 – 1.200	4 - 12	---	**

(a) EU and Codex classification

(b) Outdoor or field use (F), glasshouse application (G) or indoor application (I)
(d) internationally (GIFAP) agreed codes
(e) cfu = colony forming units
(f) according to BBCH scale; grapevines: 55 inflorescences swelling, flowers closely pressed together; 75: berries pea-sized, bunches hang; 68: 80% of flowerhoods fallen; 81: beginning of ripening: berries begin to develop variety- specific colour;
Application timing: exact timing depends on local conditions: spray when infestation will occur or according to local extension service
(g) minimum pre-harvest interval not relevant, no residues
(h) product stated as active substance expressed in colony forming units (cfu)
* spray interval max. 5 days; use product in spraying sequence with other fungicides
** spray interval 5 to 7 days, up to the day of harvest

1.5.4 Information on authorizations in EU Member States (Annex IIIB 12.1)

In Germany no plant protection product with the active substance *Bacillus subtilis* has been registered yet.

Level 2

Bacillus subtilis strain QST 713

Overall Conclusions

2 Reasoned statement of the overall conclusions

2.1 Identity

2.1.1 Identity of the micro-organism

The active substance of the biological fungicide and bactericide Serenade^{TM} WP is strain QST 713 of *Bacillus subtilis*, comprising on average 10 % by weight of the formulated product.
The species *Bacillus subtilis* strain QST 713 is a bacterium with an ellipsoidal endospore which does not extend the mother cell. The first description of the species was from Cohn (1872), by Ehrenberg (1835) formerly described as *Vibrio subtilis* (Gordon *et al.*, 1973). The genus *Bacillus* (rod-shaped, aerobic and facultative anaerobic, endospore-forming, ~ 60 species) (Priest, 1993) belongs to the family Bacillaceae (aerobic, saprophytic, endospore-forming) among the group gram-positive eubacteria (Schlegel, 1985). The occurrence of *B. subtilis* is ubiquitous in any environmental media, but primarily in soil. This strain originates from a natural, indigenous wild type, isolated from soil in California (USA) in 1995.

Using the available morphological, physiological and biochemical data, the American Type Culture Collection clearly identified the strain AQ 713 as *Bacillus subtilis*. The strain AQ 713 is identical with strain QST 713.

2.1.2 Biological properties of the micro-organism

As a saprophytic micro-organism of the soil *B. subtilis* contributes to the mineralisation of organic molecules due to break down by secreted proteases and amylases. Some of these enzymes are responsible for the occurrence of soft-rot disease caused by *B. subtilis* in several crops and are commercially exploited.

Life-cycle of *Bacillus*

All spore-formers, including members of the Genus *Bacillus*, undergo a cycle consisting of several discernible phases: germination, outgrowth, multiplication and sporulation. The primary cell formed at the end of outgrowth can, under some conditions, such as insufficient nutrients, divide asymmetrically and proceed directly to sporulation. Under favourable conditions, such as sufficient nutrients, can divide symmetrically and proceed through many divisions before sporulating.
The endospore plays a dominant role in the biology and the life-cycle of *B. subtilis* and relatives. It is a dormant structure which enables the micro-organism to survive when environmental conditions turn unfavourable for vegetative growth and is a vehicle for dispersal by dust and air streams, as it is easily blown up. The global distribution of *Bacillus* spp. may largely be derived from the endospore-forming capability. Basically the endospore is the most heat tolerant bacterial life-form, enduring temperatures >80 °C or even >100 °C. The endospore does not present an obligate stage in the life-cycle, vegetative growth by cell-division may be predominant - or even the norm, unless e.g. lack of nutrients occurs.
In a dry state endospores can remain viable for several years.

B. subtilis is not characterised by a distinct host specificity since growth is not dependent upon a host but upon supply with decomposable organic matter. The endospore is the

prevalent stage of *B. subtilis* in all environmental compartments. *B. subtilis* is not geographically restricted.

Environmental requirements

Generally *B. subtilis* reproduces under aerobic conditions, although in the presence of glucose and nitrate anaerobic growth occurs. *B. subtilis*, together with closely related species, is primarily a soil inhabitant, particularly in low-nutrient soils and is dominating the restricted micro-flora of soils with low organic content. The reported predominance of *B. subtilis* and related Bacilli in soils may indicate low demands for nutrition of these bacteria or more probably the successful survival strategy rendered by sporulation. *B. subtilis* is reported to occur predominantly in the resting stage (endospore), unless fresh organic matter has been supplied to the soil. In any case, application of organic matter, e.g. manure, will support growth of existing *B. subtilis* populations.

The influence of pH on growth of *B. subtilis* was tested by Sneath (1986). The pH-range for growth was found to be pH 5,5 to 8,5. The ATCC report states growth of *B. subtilis* strain QST 713 at pH 4.5 and pH 6. In the Voges-Proskauer test the strain QST 713 growth at pH 6.6 – 7.5. The temperature range is defined for growth of various *B. subtilis* strains: maximum growth was achieved at 45 – 55 °C; the minimum temperature allowing growth was 5 to 20 °C. It is confirmed 55 °C as the maximal temperature permitting growth of strain QST 713 of *B. subtilis*, the lowest temperature allowing growth tested was 15 °C. In liquid cultivation of *B. subtilis* optimal growth occurred at 37 to 42 °C.

In conclusion several references indicate that *B. subtilis* will survive under a broad spectrum of environmental conditions. No negative impacts on Serenade efficacy in controlling crop diseases are anticipated under normal European growing season conditions (~ 10 to 30 °C).

Relationships to known plant or animal or human pathogens

B. subtilis and close relatives are regarded as non-pathogenic micro-organisms accepted as "organism GRAS" (generally regarded as safe) by the U.S. Food and Drug Administration, while other species of the Genus *Bacillus* are known as toxin forming pathogens of vertebrates and arthropods:

B. anthracis	causes anthrax in humans and animals
B. cereus	causes gastroenteritis (via food) and opportunistic infections
B. thuringiensis	acts as an insect pathogen

Purity checks of the Technical product of *B. subtilis* strain QST 713 are continuously performed to exclude the presence of the above mentioned species.

Purity control also is applied to the manufacturing process of the formulated product Serenade™ WP Broth and WP samples were determined to be 100 % pure for *B. subtilis* and did not contain detectable levels of human pathogens or contaminant micro-organisms.

In conclusion more information are necessary for the morphological differentiation of *B. subtilis* and the above indicated pathogenic *Bacillus* species.

Genetic stability

Taken together, available knowledge indicates that gene transfer within *B. subtilis* or between *B. subtilis* and related species under natural conditions may be a rare event but can not be fully ruled out. In case of strain QST 713 this is not a problem because fermentation is started with pure cultures of QST 713. A transfer to QST 713 of genes governing undesirable properties can therefore be ruled out. Regarding a possible gene transfer after application it must be considered that QST 713 does not have undesirable trails, e.g. pathogenicity to humans, animals or plants.

Presence of secondary metabolites

B. subtilis produces different exo-enzymes contributing to the decay of organic matter. The extracellular enzyme *subtilisin* is known to elicit allergic or hypersensitive reactions in individuals repeatedly exposed to it. However according to EPA (1997) *B. subtilis* does not produce significant quantities of extracellular enzymes or toxins and *B. subtilis* appears to have low degree of virulence to humans. The results of the submitted toxicological studies on rodents do not show evidence of toxin production of QST 713 strain of *B. subtilis* at relevant levels.

Antibiotics

Different antibiotic molecules were identified as products from different strains of *B. subtilis*. Antibiotic production is specifically related to the growth stage or age of a culture. It is often associated with the end of the logarithmic growth phase and the early stages of sporulation without being directly involved in sporulation.
Several antibiotics produced by certain strains of *B. subtilis* are described, e.g. bacillomycin, bacillin, eumycin, fungistatin, iturin and mycobacillin. Numerous studies concentrated on one group of antibiotics, the iturins. Iturins consist of a cyclic octapeptide with a lipophilic β-amino acid side-chain of variable length, reported to directly interact with the cytoplasmic membrane and to be important for the displayed antifungal activities.

Other antibiotics were defined as macrocyclic polyene lactone phosphate esters, isolated from fermentation broth of certain strains of *B. subtilis*; these antibiotics were shown to act antibacterial and to act against human pathogens. However, no antibiotics used in human or animal medicine are known to be produced by *B. subtilis*.
The applicant stated that no resistance genes against antibiotics which are used in animal or human medicine are inherent to strain QST 713 of *B. subtilis*.

In conclusion more information are necessary regarding resistance of strain QST713 against antibiotics and about the antibiotics produced by it.

2.1.3 Details of uses and further information

2.1.3.1 Details of uses

The intended uses of Serenade™ WP envisaged are in viticulture, pome fruit, stone fruit in the field and lettuce under field conditions and protected cultivation as well. The application rates per treatment for orchards is 5-15 kg/ha, in viticulture and lettuce 4-12 kg/ha. The maximum numbers of application are 16 in apple and pears against scab, but only 4 against fire blight. In stone fruit 10 and in viticulture 8 applications are envisaged. In the case of let-

tuce there is no number of application given because after planting the fungicide agent should be sprayed in an interval of 5 to 7 days up to the day of harvest. In all cases the application method is spraying at infestation or according to local extension service. In the case of the control of fire blight the time of application is during blossom.

2.1.3.2 Further information

Information on handling, storage, transport or fire, destruction or decontamination, and emergency measures for the active substance as manufactured and information on packaging, cleaning procedures, handling, storage, transport or fire, emergency measures, and procedures for destruction or decontamination for the plant protection product have been supplied and are acceptable.

2.1.4 Classification and labelling

With regard to environmental fate and behaviour no EU-criteria for classification and labelling are available.

Micro-organism

The following is proposed in accordance with the latest classification and labelling guidance under Directive 67/548/EEC (i.e. in the 18th ATP published as Directive 93/21/EEC):

***Bacillus subtilis* (strain QST 713)**

Hazard symbol:	Xi	
Indication of danger:	Irritant	
Risk phrases:	R 43	May cause sensitisation by skin contact
Reasons for classification		
For justification of R 43 see	B.6.1.1.6	Absence of any data on skin sensitisation by the active ingredient
and		
	B.6.7.3	Skin sensitisation of the formulation

Preparation

The following is proposed in accordance with Directive 78/631/EEC in combination with the latest classification and labelling guidance under Directive 67/548/EEC (i.e. in the 18th ATP published as Directive 93/21/EEC):

QST 713 WP (Serenade™ WP)

Hazard symbol:	Xi	
Indication of danger:	Irritant	
Risk phrases:	R 43	May cause sensitisation by skin contact

Reasons for classification
For justification of R 43 see B.6.7.3 Skin sensitisation

2.2 Methods of Analysis

2.2.1 Analytical methods for the identification of the micro-organism

Adequate methodology exists for identification of strain QST 713 as *Bacillus subtilis* in the fermentation broth, the technical product, the plant protection product, environmental media (soil, water) and animal tissue. Analytical procedures used to determine the quality of the batches were provided (Determination of moisture content or dry weight and of content of active ingredient, tests for microbial contaminants, detection of human pathogens).

In principle *B. subtilis* is identified and analysed using biological methods, i.e. plating on microbiological media. The central criterion for identification of *Bacillus* species is the endospore-morphology. For identification of different species additional criteria are microscopic appearance, colony morphology, biochemical and physiological characteristics.
In addition, cultures on agar are used for microscopical and macroscopical identification of extraneous micro-organisms.

2.2.2 Analytical methods for formulation analysis

Serenade^{TM} WP consists of the QST strain of dried *B. subtilis*, residual fermentation media and inert ingredients. Analytical procedures used to determine the quality of the formulation were provided (Determination of moisture content and bulk density of WP and broth dry weight, content of active ingredient (cfu) for broth and WP, determination of human pathogens and other contaminants is performed on the broth of each fermentation run (batch)).

2.2.3 Methods to determine and quantify residues (viable and non-viable) of the active organisms and relevant secondary metabolites (especially toxins) on and/or in crop, foodstuffs and feeding stuffs, in animal and human body tissues and fluids, in soil, in water (including drinking water, ground water and surface water) and in air where relevant

The ingredients of Serenade^{TM} WP are inert, non-toxic and impose no environmental or health risk. Thus, it can be concluded that information about behaviour of the active ingredient, *B. subtilis*, also applies to the formulated product.
Taking into account that the active substance, *B. subtilis*, is a non-pathogenic, non-hazardous micro-organism of ubiquitous distribution, and regarding the non-reactive, non-toxic properties of all ingredients comprising the product, no residues relevant to the safety of consumers, workers or the environment do occur. Therefore, the determination of residues are not required.

2.3 Impact on human and animal health

2.3.1 Effects having relevance to human and animal health arising from exposure to the micro-organism or to impurities contained in the organism, its residual traces and metabolites

2.3.1.1 Acute toxicity, pathogenicity and infectivity

The acute toxicity and primary irritation studies with QST 713 Technical, containing *Bacillus subtilis* with residual fermentation media, are summarized in Table B.2.3-1.

Table B.2.3-1: Summary table of acute toxicity and primary irritation studies

Oral				
Species	Vehicle	Sex	NOEL (cfu/animal)	LD_{50} (cfu/animal)
Rat	Sterile water	3 per group/ sacrifice day/ sex	$> 1.13 \times 10^8$	$> 1.13 \times 10^8$
Intratracheal				
Species	Vehicle	Sex	NOEL	LD_{50} (cfu/animal)
Rat	Sterile water	5 per group/ sacrifice day/ sex	Not determined	$> 1.2 \times 10^8$
Primary dermal irritation				
Species	Vehicle	Sex	NOEL	LD_{50}
Rabbit	0.3 ml saline	3 males/3 females	Not relevant (non-irritant)	Not relevant (non-irritant)
Primary eye irritation				
Species	Vehicle	Sex	NOEL	LD_{50}
Rabbit	Moistened with sterile water	3 males/3 females	Not relevant (non-irritant)	Not relevant (non-irritant)
Acute dermal				
Species	Vehicle	Sex	NOEL	LD_{50} (cfu/animal)
Rabbit	Sterile water	5 males/5 females	Not determined	$> 2.3\text{-}2.7 \times 10^{11}$
Acute intravenous				
Species	Vehicle	Sex	NOEL (cfu/ animal)	LD_{50} (cfu/animal)
Rat	Sterile water	3 rats per group/ sacrifice day/ sex	$> 9.4 \times 10^6$	$> 9.4 \times 10^6$

The active substance, *Bacillus subtilis*, has no toxic or clinical effects after oral, intravenous or dermal administration to rats. Very slight irritating effects were recorded after skin exposure and following application to the eye of rabbits but symptoms did not imply a classification according to the relevant EC directive 67/548/EEC. This also applies to the intratracheal challenge, which caused generally minor and mostly short-termed symptoms but no deaths or gross lesions at final necropsy.
A skin sensitisation test was performed with the preparation, Serenade™ WP. Based on the sensitizing potential of the formulation and the absence of data on skin sensitisation by the active ingredient, QST 713 Technical is classified as a skin sensitiser (R43).

2.3.1.2 Genotoxicity

The genotoxic potential of the ubiquitous bacteria *Bacillus subtilis* has not been determined since there is strong evidence for a very low, or non-existent genotoxic potential of this species in the literature, specified by submitted study reports on QST 713 strain of *Bacillus subtilis*, which does not produce any genotoxic substance.

Considering the low risk potential of *Bacillus subtilis* and its ubiquitous distribution, even in foods, genotoxicity testing appeared to be dispensable.

2.3.1.3 Cell-culture studies

No cell culture studies were performed since *Bacillus subtilis*, as a natural soil inhabitant, does not enter the cytoplasm to replicate intracellularly. The members of the species *Bacillus subtilis* do not show specific attachment mechanisms typically found in organisms capable of colonizing humans.

2.3.1.4 Short-term toxicity, pathogenicity and infectivity

No relating study has been performed so far in consistence with the results of the above cited acute toxicity studies proving the absence or minor significance of clinical signs. A potential escape of the host defence mechanisms after repeated doses is also unlikely since following an intravenous administration of QST 713 Technical to rats no adverse effects became apparent over the 35-day observation period.

Nontheless, a repeated dose inhalation study is required by the Rapporteur with regard to the medical information that *Bacillus subtilis* had been isolated in some cases of food poisoning and from human infections in very few patients with a compromised immune status. Furthermore, it should be taken into account that toxicologically relevant substances (e.g., subtilisin) may be produced and that the clearance of *Bacillus subtilis* spores from some rat tissues after intratracheal and intravenous administration was rather slow.

2.3.1.5 Pathogenicity and infectivity under immunosuppression

This micro-organism is able to grow at temperatures higher than 32°C as given in the human body but it is known as usually non-pathogenic. In some cases, *Bacillus subtilis* was isolated from surgical wound or tumor drainages, but it remained locally restricted and did not influence the course of wound healing. Incidents of progressive dissipating bacterial infections caused by *Bacillus subtilis* (among other species). were only reported for highly immuno-deficient patients suffering e.g. from leukemia.
These findings suggest that under normal health conditions no pathogenicity and infectivity of *Bacillus subtilis* is expected to occur. Furthermore, the given permanent ambient exposure towards this ubiquitous bacteria must be taken into consideration. It can be assumed that the number of micro-organisms challenging an individual must be very large and the immune status of this individual very poor to facilitate an infection with *Bacillus subtilis*. The former situation might be given for a limited number of workers and operators wich can be assumed to be in a sufficient general health state whereas the latter one would apply to some persons among the general population who are usually not expected to be exposed to a high number of bacteria of this species.

2.3.1.6 Medical data

The EPA (1997) concluded that *Bacillus subtilis* is not a frank human pathogen, nor is it toxigenic like some other members of the genus. The virulence characteristics of the micro-organism are low, but on several occasions *Bacillus subtilis* has been isolated from human infections. Moreover, there have been several reported cases of food poisoning attributed to large numbers of *Bacillus subtilis* contaminated food. *Bacillus subtilis* could be expected to temporarily inhabit the skin and gastrointestinal tract of humans, but, according to the available data, it is doubtful that this organism would colonize other sites in the human body.
Bacillus subtilis produces an extracellular toxin known as subtilisin which has very low toxigenic properties but is capable of causing allergic reactions in individuals who are repeatedly exposed to it.

For the strain QST 713 of *Bacillus subtilis*, no adverse effects on human health were reported to having occurred in the laboratories and production facilities of the applicant. No specific clinical signs or poisoning symptoms can be attributed to this strain, accordingly no special therapeutic regimes can be recommended for this non-toxic and non-pathogenic micro-organism.

2.3.2 ADI

Bacillus subtilis is a micro-organism which belongs to our natural environment and is considered to be a non-pathogen for humans. In the absence of any significant evidence for toxicity, pathogenicity or infectivity of the strain QST 713 of *Bacillus subtilis* in animal studies it is neither possible nor necessary to establish an ADI.

2.3.3 AOEL

For the above mentioned reasons (2.3.2) the establishment of an AOEL is not necessary.

2.3.4 Drinking water limit

Not relevant.

2.3.5 Impact on human or animal health arising from exposure to the micro-organism or to impurities contained in the organism, its residual traces and metabolites

The potential exposure for operators was estimated for the intended uses of QST 713 WP (Serenade WP; 5 x 10^9 cfu/g). The calculation using the German model and data for worst case conditions results in estimated total exposures in a range of 2-3 mg as/kg bw/d (without PPE) corresponding to 1-1.5 x 10^8 cfu/kg bw/d. The dermal route is the predominant one although a relevant dermal absorption of the bacillus is not expected. Only about 1/100 of the total exposure would be by inhalation (~ 1 x 10^6 cfu/kg bw/d).
In relation to the results of the available acute toxicity studies, sufficient margins of safety do exist although not in all cases clear no effect levels could be demonstrated. In the intratracheal study with the active ingredient, the complete clearance from all tissues was estimated to take

a time of >100 days, however, no evidence of germination or vegetative growth was obtained. Short term toxicity studies are not available so far and should be required, therefore.
Comparing the estimated exposure in relation to the available toxicity data and according to the biological properties of *Bacillus subtilis* strain QST 713, harmful effects on the health of operators, workers or bystanders are not to be expected when the products are used in accordance with good plant protection practice. Nevertheless, due to the possible sensitising potential of the active ingredient and the sensitising properties of the product, suitable personal protective equipment is necessary, especially when handling the undiluted product and post registration surveillance should be considered.
In view of the recommended uses and application techniques, no harmful effects on the health of domestic or wild animals are assumed.

2.4 Residues

2.4.1 Definition of the residues

Residues of *B. subtilis* strain QST 713 on crops, feedingstuffs or foodstuffs are not expected at relevant concentrations:

- With regard to its natural global distribution and non-pathogenic character *B. subtilis* cells left on the surface of treated areas or plant products do not imply health or environmental impacts.
- *B. subtilis* has been used for enzyme production on a large industrial scale, and is even used for food production without having caused health or environmental hazards or damages.
- *B. subtilis* does not produce toxins.
- *B. subtilis* has no special attachment ability to plants or plant products, i.e. there is no compatibility comparable to host-pathogen interactions.
- The unfavourable environmental conditions prevailing on the leaf surface and the dependence of *B. subtilis* on organic matter supply are restricting its growth, as shown in the submitted study report (Yuan and Heins, 2000). In addition, in processing of raw products no growth or sporulation of *B. subtilis* is expected to occur.
- A plant product (fruit) carrying a layer built up of *B. subtilis* can easily be washed with water prior to consumption or juice production.

Therefore, a residue definition is not necessary

2.4.2 Residues relevant to consumer safety

A residue definition relevant to consumer safety is not necessary (see 2.4.1).

2.4.3 Residues relevant to worker safety

A residue definition relevant to worker safety is not necessary (see 2.4.1).

2.5 Fate and behaviour in the environment

2.5.1 Definition of the residues relevant to the environment

The active substance, *B. subtilis*, is a member of the natural micro-flora in soils and occurs without geographically restriction in almost any environmental niche, including the immediate environment of humans. Therefore any proliferation of introduced *B. subtilis* cells will not impede the natural micro-flora. In addition, growth of this species is dependant on supply with fresh organic matter (see chapter 2.1.2).
With regard to environmental concern of the deliberate release of micro-organisms usually the number of introduced organisms declines rapidly (sometimes after a brief period of proliferation) following application to both the soil and the field.
The available information about the behaviour of the active ingredient, *B. subtilis* QST 713, also applies to the formulated product. All inert ingredients of Serenade^{TM} WP are non-toxic and impose no environmental or health risk.

2.5.2 Fate and behaviour in soil

The phenomenon of fast decreasing vegetative cell numbers is reported for a *B. subtilis* strain introduced into soil, while in parallel sporulation increased. After a few days the cell population was shown to be stabilised as endospores. A similar population dynamic of *B. subtilis* cells applied to the soil surface at a level of 3,5 x 10^7 cfu/g was found: an initial decrease in cell numbers was followed by a stabilisation at levels of ~ 3-5 x 10^3 cfu/g. In this study neither the kind of soil (loamy sand and silty loam) nor the rhizosphere of cropped soil had an influence on the dynamics.
Another reference from the open literature showed that *B. subtilis* cells added to acid forest soil did not grow unless fungal growth took place and therefore it was concluded that the prime factor was nutrient supply; however an indirect effect of altered pH could not be excluded.
The supply with fresh organic matter appears to be a key parameter for survival of vegetative cells of *B. subtilis* introduced into soil, while the prevailing form in soil appears to be the endospore. Available information indicates, that application of organic matter, e.g. manure, will enhance growth of existing *B. subtilis* populations. The cells will produce endospores unless organic matter, e.g. manure, is supplied.

B. subtilis is an autochthonous soil micro-organism, the strain QST 713 has originally been isolated from soil in California, U.S.A.. Therefore its possible multiplication in this natural habitat does not disturb the natural micro-flora. As vegetative growth declines as the nutrient source declines this species does not seem to compete well for limited resources and *B. subtilis* populations will be subject to competition in the natural micro-flora on ecological basics. Since *B. subtilis*, including this strain, is facultative anaerobic or micro-aerophilic, growth will prevail in the superficial, aerated soil layer. Translocation of *B. subtilis* into deeper soil layers has been shown to occur at low levels.
Finally, introduced *B. subtilis* cells are not expected to exceed the natural level permanently.

Predicted load of colony forming units (cfu) on treated areas:

Serenade^{TM} WP is applied to the foliage at a rate of 15 kg/ha in maximum per treatment. An amount of 15 kg/ha corresponds to 7.5 x 10^{13} cfu/ha. Assuming the whole amount would

reach the soil surface uniformly, the resulting surface load would be approximately 7.5 x 10^5 cfu/cm^2 per treatment. After maximal 16 applications in orchards the resulting surface load would be approximately 1.2 x 10^7 cfu/cm^2.
Employing a more realistic scenario under consideration of drift results in even lower levels of surface load: In consideration that a rate of 50 % of the applied amount of product will reach the soil surface one square cm of surface will receive a theoretical load of 6 x 10^6 cfu. The maximal predicted environmental concentration would be 8 x 10^5 colony forming units per gram soil (Table 2.5-1).
This still overestimated value can be regarded as low in view of the generally high population densities of Bacilli, which occur at levels of 10^6 to 10^7 cfu/g (EPA, 1997, BOD2000-1366).
Considering the above cited references it can be expected that part of the cells reaching the soil will not survive and the residual cells will form endospores, unless fresh organic matter is supplied.

Table 2.5-1: Predicted environmental concentration (PEC)

Crop	Product application rate [kg/ha] min max	Number of applications min max	Interception	PEC [mg product/kg]	Colony forming units [cfu/g soil]
Orchards	5 - 15	16	50 %	max. 160	max. 8 x 10^5
	5 - 15	1	50 %	max. 10	max. 5 x 10^4

2.5.3 Fate and behaviour in water

B. subtilis is frequently occurring in different aquatic environments, as fresh water, estuarine and coastal waters, and endospores have been detected in sediments and even in the open ocean. However, *B. subtilis* is not regarded as an autochthonous inhabitant of aquatic environments and does not find optimal conditions for growth, e.g. waters are poor in organic C. Therefore, proliferation is not likely to occur. Bacterial cells and especially endospores may survive, but will be subject to natural competition in the diverse micro-flora of natural waters. Survival of introduced QST 713 strain of *B. subtilis* will not cause any environmental or health impact.

2.5.4 Fate and behaviour in air

Endospores are suitable for aerial distribution as they are easily blown about by wind. Therefore, under conditions of use drift spacious transport may occur.
Multiplication of *B. subtilis* in the air, aerosols or clouds can be excluded due to lack of organic matter supply and lack of mineral matrix to adhere to. In addition, during aerolisation vegetative cells of *B. subtilis* are exposed to severe environmental stress factors (desiccation, UV-radiation, temperature), therefore survival of vegetative cells is limited.

2.6 Effects on non-target species

2.6.1 Effects on terrestrial vertebrates

A 5-day acute oral toxicity and pathogenicity test with bobwhite quail showed that QST 713 technical has a low toxicity to birds. The LD50 is >5000 mg/kg/d equivalent to $>10^{11}$ cfu/kg/d.

There is no evidence of pathogenicity or replication of the QST 713 strain of *Bacillus subtilis* for birds; the same is true for mammals. Thus the intended use of *Bacillus subtilis* should not pose a risk to terrestrial vertebrates.

2.6.2 Effects on aquatic species

Studies with fish (*O. mykiss*), daphnia (*D. magna*) and algae (*S. subspicatus*) were submitted. The results indicate that QST 713 technical is of low toxicity to aquatic organisms. Gross necropsy of the fish at test end showed no signs of infection in gill, intestine or muscle tissue. All toxicity values exceed the limit value for toxic or adverse effects. Therefore a hazard classification or specific labelling according to Directive 67/548/EEC is not necessary. All TER-values are well above the relevant Annex VI trigger, even under the assumption of worst case conditions. In conclusion, the overall risk to aquatic organisms is considered to be acceptable.

2.6.3 Effects on bees

Bacillus subtilis is an ubiquitous bacterium in the environment (detected in /on soil, water, plant material, animals, food stuff). *B. subtilis* is used for control of fire blight (*Erwinia amylovora*), scab (*Venturia* spp.), monilia, bunch rot (*Botrytis cinerea*), powdery mildew (*Uncinula necator*) and downy mildew. Honeybees may be contaminated by application of *B. subtilis* products in fruit. The LC_{50} for oral uptake of *B. subtilis* strain QST 713 was 8900 ppm. This corresponds to 15 times the maximum application rate which indicates a minimum risk for honeybees by the practical use of formulated products containing B. *subtilis* strain QST 713.

2.6.4 Effects on other arthropod species

According to the data submitted some rather low inherent toxicity was demonstrated in basic laboratory tests. No signs of pathogenecity and infectivity of formulated *Bacillus subtilis* were reported. Sublethal effects found were judged negligible, because only single individuals were affected. However, no dose-response-relationship was apparent. The effects reported are considered acceptable.

It is therefore established in the light of current scientific and technical knowledge and as laid down in the SETAC/ESCORT *"Guidance document on regulatory testing procedures for pesticides with non-target arthropods*" (Barrett et al., 1994), that the use of *Bacillus subtilis* as outlined in this monograph has no unacceptable influence on non-target arthropods, represented by species of three ecological groups.

2.6.5 Effects on earthworms and other soil macro-organisms

No data have been submitted.

Bacillus subtilis is an autochthonous micro-organism of the soil. However, effects of strain QST 713 on earthworms can not be excluded, as there is no information on the natural occurrence of this specific strain in soil. Application of the formulated product also might enhance the potential exposure concentration for earthworms. In addition, there are indications from a study on mice where two out of four tested strains exhibited toxicity after intraperitoneal injection.

The possible risk of *Bacillus subtilis* strain QST 713 should be addressed by conducting an acute study combined with a histopathological investigation of the earthworm tissue.

2.6.6 Effects on other non-target organisms (additional studies)

B. subtilis is a viable micro-organism of ubiquitous occurrence. The phenomenon of fast decreasing vegetative cell numbers is reported for a *B. subtilis* strain introduced into soil, while in parallel sporulation increased. After a few days the cell population was shown to be stabilised as endospores.
As vegetative growth declines as the nutrient source declines this species does not seem to compete well for limited resources and *B. subtilis* populations will be subject to competition in the natural microflora.
Finally, introduced *B. subtilis* cells are not expected to exceed the natural level permanently.

2.6.7 Effects on biological methods of sewage treatment

Vegetative growth of *Bacillus subtilis* in water is not expected. Therefore effects on biological methods of sewage treatment are not anticipated.

Appendix 1

Bacillus subtilis strain QST 713

Standard Terms and Abbreviations

2.7 Appendices

2.7.1 Appendix I: Standard terms and abbreviations

Part 1 Technical Terms

A	ampere
ACH	acetylcholine
AChE	acetylcholinesterase
ADI	acceptable daily intake
ADP	adenosin diphosphate
AE	acid equivalent
AFID	alkali flame-ionization detector or detection
A/G	albumin/globulin ratio
ai	active ingredient
ALD_{50}	approximate median lethal dose, 50 %
ALT	alanine amitrotransferase (SGPT)
AMD	automatic multiple development
ANOVA	analysis of variance
AOEL	acceptable operator exposure level
AP	alkaline phosphatase
approx	approximate
ARC	anticipated residue contribution
ARfD	acute reference dose
as	active substance
AST	aspartate aminotransferase (SGOT)
ASV	air saturation value
ATP	adenosine triphosphate
BCF	bioconcentration factor
bfa	body fluid assay
BOD	biological oxygen demand
bp	boiling point
BSAF	biota-sediment accumulation factor
BSE	bovine spongiform encephalopathie
BSP	bromosulfophthalein
Bt	bacillus thuringiensis
Bti	bacillus thuringiensis israelensis
Btk	bacillus thuringiensis kurstaki
Btt	bacillus thuringiensis tenebrionis
BUN	blood urea nitrogen
bw	body weight
c	centi- (x 10^{-2})
°C	degree celsius (centigrade)
CA	controlled atmosphere
CAD	computer aided design
CADDY	computer aided dossier and data supply (an electronic dossier interchange and archiving format)
cd	candela

CDA	controlled drop(let) application
cDNA	complementary DNA
CEC	cation exchange capacity
cf	confer, compare to
CFU	colony forming units
ChE	cholinesterase
CI	confidence interval
CL	confidence limits
cm	centimetre
CNS	central nervous system
COD	chemical oxygen demand
CPK	creatinine phosphatase
cv	coeficient of variation
Cv	ceiling value
CXL	Codex Maximum Residue Limit (Codex MRL)
d	day
DES	diethylstilboestrol
DFR	dislodgeable foliar residue
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
dna	designated national authority
DO	dissolved oxygen
DOC	dissolved organic carbon
dpi	days pot inoculation
DRES	dietary risk evaluation system
DT_{50}	period required for 50 percent dissipation (define method of estimation)
DT_{90}	period required for 90 percent dissipation (define method of estimation)
dw	dry weight
DWQG	drinking water quality guidelines
ε	decadic molar extinction coefficient
EC_{50}	effective concentration
ECD	electron capture detector
ECU	European currency unit
ED_{50}	median effective dose
EDI	estimated daily intake
ELISA	enzyme linked immunosorbent assay
e-mail	electronic mail
EMDI	estimated maximum daily intake
EPMA	electron probe micro analysis
ERC	environmentally relevant concentration
ERL	extraneous residue limit
F	field
F_0	parental generation
F_1	filial generation, first
F_2	filial generation, second
FIA	fluorescence immuno assay
FID	flame ionization detector
FOB	functional observation battery
fp	freezing point

FPD	flame photometric detector
FPLC	fast protein liquid chromatography
g	gram
G	glasshouse
GAP	good agricultural practice
GC	gas chromatography
GC-EC	gas chromatography with electron capture detector
GC-FID	gas chromatography with flame ionization detector
GC-MS	gas chromatography-mass spectrometry
GC-MSD	gas chromatography with mass-selective detection
GEP	good experimental practice
GFP	good field practice
GGT	gamma glutamyl transferase
GI	gastro-intestinal
GIT	gastro-intestinal tract
GL	guideline level
GLC	gas liquid chromatography
GLP	good laboratory practice
GM	geometric mean
GMO	genetically modified organism
GMM	genetically modified micro-organism
GPC	gel-permeation chromatography
GPPP	good plant protection practice
GPS	global positioning system
GSH	glutathion
GV	granulosevirus
h	hour(s)
H	Henry`s Law constant (calculated as a unitless value) (see also K)
ha	hectare
Hb	haemoglobin
HCG	human chorionic gonadotropin
Hct	haematocrit
HDT	highest dose tested
hL	hectolitre
HEED	high energy electron diffraction
HID	helium ionization detector
HPAEC	high performance anion exchange chromatography
HPLC	high pressure liquid chromatography or high performance liquid chromatography
HPLC-MS	high pressure liquid chromatography – mass spectrometry
HPPLC	high pressure planar liquid chromatography
HPTLC	high performance thin layer chromatography
HRGC	high resolution gaschromatography
Hs	Shannon-Weaver index
Ht	haematocrit
I	indoor
I_{50}	inhibitory dose, 50 %
IC_{50}	median immobilization concentration
ICM	integrated crop management

ID	ionization detector
IEDI	international estimated daily intake
IGR	insect growth regulator
im	intramuscular
inh	inhalation
ip	intraperitoneal
IPM	integrated pest management
IR	infrared
ISBN	international standard book number
ISSN	international standard serial number
iv	intravenous
IVF	in vitro fertilization
k	kilo
K	Kelvin or Henry`s Law constant (in athmospheres per cubic meter per mole) (see also H)13
K_{ads}	adsorption constant
K_{des}	apparent desorption coefficient
K_{oc}	organic carbon adsortion coefficient
K_{om}	organis matter adsorption coefficient
kg	kilogram
L	litre
LAN	local area network
LASER	light amplification by stimulated emission
LBC	loosely bound capacity
LC	liquid chromatography
LC-MS	liquid chromatography-mass spectrometry
LC_{50}	lethal concentration, median
LCA	life cycle analysis
LCLo	lethal concentration low
LC-MS-MS	liquid chromatography with tandem mass spectrometry
LD_{50}	lethal dose, median; dosis letalis media
LDLo	lethal dose low
LDH	lactate dehydrogenase
LOAEC	lowest observable adverse effect concentration
LOAEL	lowest observable adverse effect level
LOD	limit of determination
LOEC	lowest observable effect concentration
LOEL	lowest observable effect level
LOQ	limit of quantification (determination)
LPLC	low pressure liquid chromatography
LSC	liquid scintillation counting or counter
LSD	least squared denominator multiple range test
LSS	liquid scintillation spectrometry
LT	lethal threshold
m	metre
M	molar
µm	micrometer (micron)
MC	moisture content
MCH	mean corpuscular haemoglobin

MCHC	mean corpuscular haemoglobin concentratin
MCV	mean corpuscular volume
MDL	method detection limit
MFO	mixed function oxidase
µg	microgram
mg	milligram
MHC	moisture holding capacity
min	minute(s)
mL	millilitre
MLT	median lethal time
MLD	minimum lethal dose
mm	millimetre
mo	month(s)
mol	Mol
MOS	margin of safety
mp	melting point
MRE	maximum residue expected
MRL	maximum residue limit or level
mRNA	messenger ribonucleic acid
MS	mass spectrometry
MSDS	material safety data sheet
MTD	maximum tolerated dose
n	normal (defining isomeric configuration)
NAEL	no adverse effect level
nd	not detected
NEDI	no effect daily intake (mg/kg body wt/day)
NEL	no effect level
NERL	no effect residue level
ng	nanogram
nm	nanometer
NMR	nuclear magnetic resonance
no	number
NOAEC	no observed adverse effect concentration
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
NOED	no observed effect dose
NOEL	no observed effect level
NOIS	notice of intend to suspend
NPD	nitrogen-phosphorus detector or detection
NPV	nuclear polyhedrosis virus
NR	not reported
NTE	neurotoxic target esterase
OC	organic carbon content
OCR	optical character recognition
ODP	ozone-depleting potential
ODS	ozone-depleting substances
OM	organic matter content
op	organophosphorus pesticide
Pa	pascal

PAD	pulsed amperometric detection
2-PAM	2-pralidoxime
pc	paper chromatography
PC	personal computer
PCV	haematocrit (packed corpuscular volume)
PEC	predicted environmental concentration
PEC_A	predicted environmental concentration in air
PEC_S	predicted environmental concentration in soil
PEC_{SW}	predicted environmental concentration in surface water
PEC_{GW}	predicted environmental concentration in ground water
PED	plasma-emissions-detektor
pH	pH-value
PHED	pesticide handler`s exposure data
PHI	pre-harvest interval
PIC	prior informed consent
pic	phage inhibition capacity
PIXE	proton inducted X-ray emission
pK_a	negative logarithm (to the base 10) of the dissociation constant)
PNEC	predicted no effect concentration
po	by mouth (per os)
P_{ow}	partition coefficient between n-octanol and water
POP	persistent organic pollutants
ppb	parts per billion (10^{-9})
PPE	personal protective equipment
ppm	parts per million (10^{-6})
ppp	plant protection product
ppq	parts per quadrillion (10-24)
ppt	parts per trillion (10^{-12})
PSP	phenolsulfophthalein
PrT	prothrombin time
PRL	practical residue limit
PT	prothrombin time
PTDI	provisional tolerable daily intake
PTT	partial thromboplastin time
QSAR	quantitative structure-activity relationship
r	correlation coefficient
r^2	coefficient of determination
RBC	red blood cell
REI	restricted entry interval
R_f	ratio of fronts
RfD	reference dose
RH	relative humidity
RL_{50}	residual lifetime
RNA	ribonucleic acid
RP	reversed phase
rpm	reversed phase material
rRNA	ribosomal ribonucleic acic
RRT	relative retention time
RSD	relative standard deviation

s	second
SAC	strong adsorption capacity
SAP	serum alkaline phosphatase
SAR	structure/activity relationship
SBLC	shallow bed liquid chromatography
sc	subcutaneous
sce	sister chromatid exchange
SD	standard deviation
SE	standard error
SEM	standard error of the mean
SEP	standard evaluation procedure
SF	safety factor
SFC	supercritical fluid chromatography
SFE	supercritical fluid extraction
SIMS	secondary ion mass spectroscopy
SOP	standard operating procedures
sp	species (only after a generic name)
SPE	solid phase extraction
SPF	specific pathogene free
spp	subspecies
sq	square
SSD	sulfur specific detector
SSMS	spark source mass spectrometry
STEL	short term exposure limit
STMR	supervised trials median residue
t	tonne (metric ton)
$t_{1/2}$	half-life (define method of estimination)
T_3	tri-iodothyroxine
T_4	thyroxine
TADI	temporary acceptable daily tntake
TBC	tightly bound capacity
TCD	thermal conductivity detector
TCLo	toxic concentration low
TID	thermionic detector, alkali flame detector
TDLo	toxic dose low
TDR	time domain reflectrometry
TER	toxicity exposure ration
TER_I	toxicity exposure ration for initial exposure
TER_{ST}	toxicity exposure ration following repeated exposure
TER_{LT}	toxicity exposure ration following chronic exposure
tert	tertiary (in a chemical name)
TEP	typical end-use product
TGGE	temperature gradient gel electrophoresis
TIFF	tag image file format
TLC	thin layer chromatography
Tlm	median tolerance limit
TLV	threshold limit value
TMDI	theoretical maximum daily intake
TMRC	theoretical maximum residue contribution

TMRL	temporary maximum residue limit
TOC	total organic chlorine
Tremcard	Transport emergency card
tRNA	transfer riboncleic acid
TSH	thyroid stimulating hormone (thyrotropin)
TWA	time weighted average
UDS	unscheduled DNA synthesis
UF	uncertainly factor (safety factor)
ULV	ultra low volume
UV	ultraviolet
v/v	volume ratio (volume per volume)
WBC	white blood cell
wk	week
wt	weight
w/v	weight per volume
w/w	weight per weight
XRFA	X-ray fluorescence analysis
yr	year
<	less than
≤	less than or equal to
>	greater than
≥	greater than or equal to

Part 2 Organisations and Publications

ACPA	American Crop Protection Association
ACTM	American Society for Testing and Materials
BA	Biological Abstracts (Philadelphia)
BART	Beneficial Arthropod Registration Testing Group
CA	Chemical Abstracts
CAB	Centre for Agriculture and Biosciences International
CAC	Codex Alimentarius Commission
CAS	Chemical Abstracts Service
CCFAC	Codex Committee on Food Additives and Contaminants
CCGP	Codex Committee on General Principles
CCPR	Codex Committee on Pesticide Residues
CCRVDF	Codex Committee on Residues of Veterinary Drugs in Food
CE	Council of Europe
CIPAC	Collaborative International Pesticides Analytical Council Ltd
COREPER	Comite des Representants Permanents
EC	European Commission
ECB	European Chemical Bureau
ECCA	European Crop Care Association
ECDIN	Environmental Chemicals Data and Information of the European Communities
ECDIS	European Environmental Chemicals Data and Information System
ECE	Economic Commission for Europe

ECETOC	European Chemical Industry Ecology and Toxicology Centre
ECLO	Emergency Centre for Locust Operations
ECMWF	European Centre for Medium Range Weather Forecasting
ECPA	European Crop Protection Association
EDEXIM	European Database on Export an Import of Dangerous Chemicals
EHC (number)	Environment Health Criteria (number)
EHCD	Environmental Health Criteria Document
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINCS	European List of New Chemical Substances
EMIC	Environmental Mutagens Information Centre
EPA	Environmental Protection Agency
EPO	European Patent Office
EPPO	European and Mediterranean Plant Protection Organization
ESCORT	European Standard Characteristics of Beneficials Regulatory Testing
EU	European Union
EUPHIDS	European Pesticide Hazard Information and Decision Support System
EUROPOEM	European Predictive Operator Exposure Model
FAO	Food and Agriculture Organization of the UN
FOCUS	Forum for the Co-ordination of Pesticide Fate Models and their Use
FRAC	Fungicide Resistance Action Committee
GATT	General Agreement on Tariffs and Trade
GAW	Global Atmosphere Watch
GCOS	Global Climate Observing System
GCPF	Global Crop Protection Federation (formerly known as GIFAP)
GEDD	Global Environmental Data Directory
GEMS	Global Environmental Monotoring System
GIEWS	Global Information and Early Warning System for Food and Agriculture
GIFAP	Groupement International des Association Nationales de Fabricants de Produits Agrochimiques (now known as GCPF)
GRIN	Germplasm Resources Information Network
HRAC	Herbicide Resistance Action Committee
IARC	International Agency for Research on Cancer
IATS	International Academy of Toxicological Science
IBT	Industrial Bio-Test Laboratories
ICBB	International Commissin of Bee Botany
ICBP	International Council for Bird Preservation
ICES	International Council for the Exploration of the Seas
ICPBR	International Commissin for Plant-Bee Relationships
ILO	International Labour Organization
IMO	International Maritime Organisation
IOBC	International Organization for Biological Control of noxious Animals and Plants
IPCS	International Programme on Chemical Safety
IRAC	Insecticide Restistance Action Committee
IRC	International Rice Commission
ISCO	International Soil Conservation Orginazation
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry

JECFA	FAO/WHO Joint Expert Committee on Food Additives
JFCMP	Joint FAO/WHO Food and Animal Feed Contamination Monitoring Programme
JMP	Joint Meeting on Pesticdes (WHO/FAO)
JMPR	Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues (Joint Meeting on Pesticide Residues)
NATO	North Atlantic Treaty Organisation
NAFTA	North American Free Trade Agreement
NCI	National Cancer Institute (USA)
NCTR	National Center for Toxicological Research (USA)
NGO	non-governmental organization
NTP	National Toxicology Programme (USA)
OECD	Organization for Economic Cooperation and Development
OLIS	On-line Information Service of OECD
PAN	Pesticides Action Network
RNN	Re-registration Notification Network
RTECS	Registry of Toxic Effects of Chemical Substances (USA)
SCPH	Standing Committee on Plant Health
SETAC	Society of Environmental Toxicology and Chemistry
SI	Systeme International d`Unites
SITC	Standard International Trade Classification
TOXLINE	Toxicology Information On-line
UN	United Nations
UNEP	United Nations Environment Programme
WCDP	World Climate Data Programmme
WCP	Workd Climate Programme
WCRP	World Climate Research Programme
WFP	World Food Programme
WHO	World Health Organization
WTO	World Trade Organization
WWF	World Wide Fund for Nature

Appendix 2

Bacillus subtilis strain QST 713

Specific Terms and Abbreviations

2.7.2 Appendix II: Specific terms and abbreviations

Abb.	Definition
PAS	pure active substance
TAS	technical active substance

Appendix 3

Bacillus subtilis strain QST 713

List of End-Points

2.7.3 Appendix III: Listing of end points

2.7.3.1 Appendix III.1: Chapter 1 (Identity and biological properties of the micro-organism)

Intended uses:	Biocontrol of plant pathogenic fungi in viticulture and horticulture

1. Identity and biological properties of the micro-organism

Known or new organism:	Bacterium *Bacillus subtilis* (Cohn 1872)
Taxonomy:	The genus *Bacillus* belongs to the family Bacillaceae among the group gram-positive eubacteria.
Species, subspecies, strain:	Strain QST 713, identical with strain AQ 713
Identification / detection:	Using the available morphological, physiological and biochemical data, the strain QST 713 was clearly identified as *Bacillus subtilis*. Besides the basically relevant positive Catalase reaction inherent to all *Bacillus* species, further biochemical key parameters identifying strain QST 713 as *B. subtilis* are e.g.: positive Voges-Proskauer reaction and growth in 7 % NaCl.
Methods of analysis:	The species is identified by microscopy using classical morphological (cellular and colonial morphology) criteria and by using physiological and biochemical criteria.
Mode of action:	The mode of action of *B. subtilis* is fungistatic and fungitoxic by disruption of hyphae following contact with the fungal pathogen at the leaf surface. Besides antagonism nutrient competition is involved in the mode of action and more importantly *B. subtilis* induces systemic resistance response of the plant, indicated by enhanced peroxidase production.
Life cycle:	All spore-formers, including members of the Genus *Bacillus*, undergo a cycle consisting of several discernible phases: germination, outgrowth, multiplication, and sporulation. The primary cell formed at the end of outgrowth can, under some conditions, such as insufficient nutrients, divide asymmetrically and proceed directly to sporulation or, in time of favourable conditions, such as sufficient nutrients, can divide symmetrically and proceed through many divisions before sporulating. The endospore plays a dominant role in the biology and the life-cycle of *B. subtilis* and relatives. It is a dormant structure which enables the micro-organism to survive when environmental conditions turn unfavourable for vegetative growth and is a vehicle for dispersal by dust and air streams, as it is easily blown up. The global distribution of *Bacillus* spp. may largely be derived from the endospore-forming capability. Basically the endospore is the most heat tolerant bacterial life-form, enduring temperatures >80°C or even >100°C. The endospore

	does not present an obligate stage in the life-cycle, vegetative growth by cell-division may be predominant - or even the norm, unless e.g. lack of nutrients occurs. In a dry state endospores can remain viable for several years.
Host specificity:	*B. subtilis* is not characterised by a distinct host specificity since growth is not dependant upon a host but upon supply with decomposable organic matter. Moreover the endospore is prevalent in all environmental compartments and *B. subtilis* is not geographically restricted.
Known opportunist:	*B. subtilis* is considered an opportunist with no pathogenic potential. In some cases *B. subtilis* was isolated from surgical wounds or tumour drainages; only highly immunosuppressed patients were reported to have suffered from dissipating infections.
Toxin production:	*B. subtilis* produces different exo-enzymes contributing to the decay of organic matter. The extracellular enzyme *subtilisin* is known to elicit allergic or hypersensitive reactions in individuals repeatedly exposed to it however its toxigenic properties are assessed to be very low. *B. subtilis* does not produce significant quantities of extracellular enzymes or toxins and is generally considered to have a low degree of virulence to humans.
Resistance:	Up to now there is no indication of decreasing efficacy of the *Bacillus subtilis* strain in Serenade™ WP against fungal pathogens to be controlled. The mode of action of strain QST 713 of *Bacillus subtilis* has been demonstrated to rely on a broader base than single site action, since it includes diverging mechanisms not easily to overcome by pathogens. The risk on the occurrence of development of resistance is to be classified as low.
Resting stages:	The endospore of *B. subtilis* is a dormant structure which enables the micro-organism to survive when environmental conditions turn unfavourable for vegetative growth and is a vehicle for dispersal by dust and air streams, as it is easily blown up.
Production control:	Each “seed”(liquid media with suspended cells) transfer is checked for purity both microscopically and by streak plating. The completed fermentation material (broth) of each fermentation run (batch) is tested by counts of colony forming units (cfu) of *B. subtilis*, microscopic examination, optical density and is tested for contaminants by plating analysis, esp. with regard to human pathogens. Content of cfu and contaminants may additionally be determined for the Technical Powder. The test results showed no detectable levels of human pathogens or other contaminants.

2.7.3.2 Appendix III.2: Chapter 2 (Hazard evaluation)

Hazard to humans

Pathogenicity:	No evidence of adverse effects from acute studies - except minor and transient effects after intratracheal challenge.
Infectivity:	No evidence of adverse effects from acute studies. *B. subtilis* infections are only reported from immuno-deficient patients.
Toxicity:	Rat LD50 oral: > 1.13 x 10^8 cfu/animal Rat LD50 intratracheal: > 1.2 x 10^8 cfu/animal Rabbit LD50 dermal > 2.3-2.7 x 10^{11} cfu/animal Rat LD50 intravenous: > 9.4 x 10^6 cfu/animal
Irritation, Sensitisation:	Rabbit: Very slight irritating effects (skin, eye) Based on the sensitising property of the formulation: R43
Genotoxicity:	Not relevant since no genotoxins produced
Medical reports:	Limited database: No adverse health effects observed among personnel involved in laboratory investigations. *B. subtilis* is capable of producing subtilisin which may cause allergic reactions after repeated exposure. *B. subtilis* has been reported to be associated with food poisoning and infections in immuno-deficient patients.
Formulation:	Rat LD50 oral: > 5000 mg/kg bw (~ 2.5 x 10^{10} cfu/kg bw) Rat LD50 inhalation: > 0.63 mg/l air; 4 h (~ 5 x 10^8 cfu/kg bw) Rabbit LD50 dermal > 2000 mg/kg bw (~ 1 x 10^{10} cfu/ kg bw) Skin sensitisation (Buehler test): positive (R43)

Effects on non-target organisms

Effects on birds (Annex IIB, point 8.1, Annex IIIB, point 10.1)

Information on toxicity, infectivity and pathogenicity	No evidence of pathogenicity or replication of the QST 713 strain of *Bacillus subtilis* in birds; 5-day-LD50 (bobwhite quail): > 10^{11} cfu/kg/d
Further information	none

Effects on aquatic organisms (Annex IIB, point 8.2, Annex IIIB, point 10.2)

Group	Test substance	Time-scale	Endpoint	Toxicity, infectivity and pathogenicity
Laboratory tests				
Oncorhynchus mykiss	Bacillus subtilis QST 713 Technical	30 d	LC_{50}	162 mg as/L (1)
Daphnia magna		48 h	EC_{50}	108 mg as/L
Scenedesmus subspicatus		72 h	NOEC	100 mg as/L

(1) *No signs of infection in gill, intestine or muscle tissue at test end.*

Effects on bees (Annex IIB, point 8.3; Annex IIIB, point 10.3):

Information on toxicity, infectivity and pathogenicity to bees	No evidence of toxicity of *Bacillus subtilis* QST 713 to honeybees; 5-day-Dietary LC_{50}: ~ 8900 ppm, equivalent to ~ 1.8 x 108 cfu/ml diet
Further information	none

Effects on arthropods other than bees (Annex IIB, point 8.4, Annex IIIB, point 10.4)

Test material	Species	Developmental stage	Substrate	Dosage kg/ha	Effects % lethal	Effects % sublethal
Predatory mites						
Bacillus subtilis (Serenade WP)	*T. pyri*	Protonymphs	I	16	30.7	13. 04
Parasitoids						
Bacillus subtilis (Serenade)	*A. rhopalosiphi*	Adults	I	16	5.13	25.3
Bacillus subtilis (Serenade WP)	*N. vitripennis*	Adults	I	0.9	46.15	
Bacillus subtilis (Serenade WP)	*N. vitripennis*	Adults	I	9	19.23	
Bacillus subtilis (Serenade WP)	*N. vitripennis*	Adults	I	90	65.39	
Plant dwelling species						
Bacillus subtilis (Serenade WP)	*C. carnea*	Larvae	I	0.9	0.5	
Bacillus subtilis (Serenade WP)	*C. carnea*	Larvae	I	9	47.6	
Bacillus subtilis (Serenade WP)	*C. carnea*	Larvae	I	90	26.3	
Bacillus subtilis (Serenade WP)	*H. convergens*	Adults	I	0.9	11.8	
Bacillus subtilis (Serenade WP)	*H. convergens*	Adults	I	9	4.7	
Bacillus subtilis (Serenade WP)	*H. convergens*	Adults	I	90	2.4	

I = Inert substrate,

Effects on earthworms

Information on toxicity, infectivity and pathogenicity to earthworms	No data available
Reproductive toxicity	

Additional studies

Bacillus subtilis is a micro-organism of ubiquitous occurrence but primarily a soil inhabitant. As vegetative growth declines as the nutrient sources declines this species does not seem to compete well for limited resources and *B. subtilis* population will be subject to competition in the natural microflora.

2.7.3.3 Appendix III.3: Chapter 3 (Exposure assessment and risk evaluation)

Operator exposure

Application method:	FCTM, HCTM, HCHH
Operator exposure models:	In relation to the results of the available acute toxicity studies, sufficient margins of safety exist (calculation on the basis of cfu / German model)

Exposure of the environment

Natural occurrence, background level:	*B. subtilius* is a ubiquitous -not geographically restricted- inhabitant of the soil, from which it is spread to associated environments, including plants and plant materials (straw, composts), foods (cereals, esp. dried spices), animals and their faeces (by ingestion of spores) and is also naturally found in aquatic environments (fresh water, estuarine and coastal waters). Although *B. subtilis* is commonly found in soil it occurs in almost any environment, including niches in kitchen and bathrooms. The magnitude of occurrence of *B. subtilis* in the soil is not definitely stated in the supplied literature. Indications for their general prevalence can be derived from high levels of presumably soil-born *Bacillus* spp. spores in straw approaching 10^5 cfu/g, and from the high numbers of *Bacillus* spp. found in coastal waters (where they constitute up to 20 % of total bacterial population) and from the major contribution of their endospores in estuarine and coastal sediments (achieving up to 80 % of the heterotrophic flora).

Consumer exposure:

Residues:	Residues of *B. subtilis* strain QST 713 on crops, feedingstuffs or foodstuffs are not expected at relevant concentrations: - With regard to its natural global distribution and non-pathogenic character *B. subtilis* cells left on the surface of treated areas or plant products do not imply health or environmental impacts. - *B. subtilis* has been used for enzyme production on a large industrial

scale, and is even used for food production without having caused health or environmental hazards or damages. - *B. subtilis* does not produce toxins. - *B. subtilis* has no special attachment ability to plants or plant products, i.e. there is no compatibility comparable to host-pathogen interactions. - The unfavourable environmental conditions prevailing on the leaf surface and the dependence of *B. subtilis* on organic matter supply are restricting its growth. In addition, in processing of raw products no growth or sporulation of *B. subtilis* is expected to occur.

Level 3

Bacillus subtilis strain QST 713

Proposal for the Decision

3 Proposed decision with respect to the application for inclusion of the active substance in Annex I

3.1 Background to the proposed decision

The data submitted concerning biological properties of the microorganism are considered almost sufficient. However, more detailed information with regard to the biological properties of *B. subtilis* strain QST 713 is considered necessary for a final evaluation. The relationships to known plant, animal or human pathogens, more information about resistance of *B. subtilis* strain QST 713 against antibiotics as well as about the antibiotics produced by *B. subtilis* strain QST 713 should be provided.

With respect to physical, chemical and biological properties of the formulated product the dossier needs to be completed as indicated in Vol. 1, point 4.1.

The available data on toxicology, pathogenicity and infectivity are not considered sufficient to allow a definitive risk evaluation of *Bacillus subtilis* (strain QST 713) in humans although all the studies which are unequivocally mandatory according to Directive 91/414/EEC have been submitted and were conducted according to guideline requirements under Good Laboratory Practice regulations.
However, due to the biological properties of *Bacillus subtilis* strain QST 713, e.g. slow clearance of *Bacillus subtilis* spores from some rat tissues after intratracheal and intravenous administration, as well as because data on the production of subtilisin are missing, a repeat-dose inhalation study and information on subtilisin production is required (see point 4.1).

No indications of relevant effects on non-target organisms could be derived from the available data. However, no data have been submitted to address the potential risk to earthworms.

3.2 Proposed decision concerning inclusion in Annex I

Due to the data gaps identified it is proposed to postpone the decision on the inclusion of *Bacillus subtilis* (strain QST 713) in Annex I of Directive 91/414/EEC.

3.3 Rational for the postponement of the decision to include the active substance in Annex I, or for the conditions and restrictions to be associated with a proposed inclusion in Annex I, as appropriate

More detailed information with regard to the biological properties of *B. subtilis* strain QST 713 is considered necessary for a final evaluation. The relationships to known plant, animal or human pathogens, more information about resistance of *B. subtilis* strain QST 713 against antibiotics as well as about the antibiotics produced by *B. subtilis* strain QST 713 should be provided.

With respect to physical, chemical and biological properties of the formulated product the dossier needs to be completed.

The available data on toxicology, pathogenicity and infectivity are considered to adequately support the risk evaluation of *Bacillus subtilis* (strain QST 713) in humans. Nevertheless,

additional data are required to make a complete assessment. Depending on the results of additional data to be submitted changes in the conditions and possible restrictions might arise.

With respect to effects on non-target organisms no data have been submitted to address the potential risk to earthworms.

Level 4

Bacillus subtilis strain QST 713

Demand for Further Information

4 Further information to permit a decision to be made, or to support a review of the conditions and restrictions associated with the proposed inclusion in Annex I

4.1 Data which are necessary for an unrestricted inclusion in Annex I of Council Directive 91/414/EEC

4.1.1 Relationships to known plant or animal or human pathogens (Annex IIB 2.6)

More information is necessary for the morphological differentiation of *B. subtilis* and the indicated pathogenic *Bacillus* species (*B. anthracis, B. cereus and B. thuringiensis*).

4.1.2 Antibiotics and other antimicrobial agents (Annex IIB 2.9)

More detailed information is necessary regarding a resistance of strain QST713 against antibiotics and about the antibiotics produced by *B. subtilis* strain QST 713.

4.1.3 Toxicology, pathogenicity and infectivity (Annex IIB 5)

1. Repeated dose study:
Based on the medical information *Bacillus subtilis* has been isolated in some cases of food poisoning and from human infections in very few patients with a compromised immune status. With regard to the production of a toxicologically relevant substance (e.g., subtilisin) and the slow clearance of *Bacillus subtilis* spores from some rat tissues after intratracheal and intravenous administration, a repeated dose inhalation study is required in this special case.

2. Information on subtilisin:
Data on the production of subtilisin by strain QST 713 of *Bacillus subtilis* and on the appropriate limits for subtilisin as set by the U.S. OSHA (Occupational Safety and Health Administration) to minimise the risk of allergic reactions in individuals repeatedly exposed to this substance should be submitted.

4.1.4 Physical, chemical and technical properties of the plant protection product

To the product: Serenade™:
IIIB 2.3: The study according to EEC method A 14 has to be submitted.
IIIB 2.4: The study according to EEC method A 10 has to be submitted.
IIIB 2.7.3: The applied method is not indicated.

4.1.5 Acute toxicity and/or pathogenicity and infectivity to earthworms (Annex IIB 8.5)

Data on acute toxicity, infectivity and pathogenicity of *Bacillus subtilis* to earthworms must be submitted.

4.2 Data which should be submitted for an assessment on Member State level

Monograph

15 May 2001

Bacillus subtilis
strain QST 713

Volume 2

Annex A

List of Tests and Studies

Rapporteur Member State: Germany

Contents

A.1 Identity (Annex IIB 1, 3.1 to 3.3; Annex IIIB 1, 3.1 to 3.7, 3.9 and 12.1)

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
Anonymous	AIIB-1.3; AIIB-2.5; AIIB-4.1	1997	Project Report SC 3817 - Bacterial Characterization ATCC. not GLP, unpublished BMF2000-56	Y	QST
Bellet, E. M.	AIIB-1.4; AIIB-2.1	1998	Manufacturing and Analytical Data for QST 713 Technical. EPA Reg. No.: 69592-X GLP, unpublished BMF2000-66	Y	QST
Cunningham Hilbig, L.	AIIB-1.4	2000	QST 713 Technical Powder - Confidential Statement of Formula. not GLP, unpublished BMF2000-64	Y	QST
Cunningham Hilbig, L.	AIIIB-1.4	1999	MANUFACTURING PROCESS FOR SERENADE WP FROM QST 713 STRAIN OF DRIED BACILLUS SUBTILIS. not GLP, unpublished PHY2000-714	Y	QST
Cunningham Hilbig, L.	AIIIB-1.4	2000	SERENADE WP-CONFIDENTIAL STATEMENT OF FORMULA. not GLP, unpublished PHY2000-715	Y	QST
Gingras, B. A.	AIIB-1.4	1998	Lot Characterisation of Bacillus subtilis strain QST 713. L08726 SN1 GLP, unpublished BMF2000-65	Y	QST
Gingras, B. A.	AIIIB-1.4	1998	LOT CHARACTERIZATION OF QST 713STRAIN OF DRIED BACILLUS SUBTLS WITH RESIDUAL FERMENTATIO MEDIA IDENTIFIED AS QST 713 WP. GLP, unpublished PHY2000-716	Y	QST

[1] Only notifier listed

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[2]
Gordon, R. E.	AIIIB-1.4	1973	THE GENUS BACILLUS. not GLP, published 427, 1973, pp.36-253 PHY2000-717	N	-
Gordon, R.E., Haynes, W.C. and Pang, C.H.-N.	AIIB-1.3; AIIB-2.1; AIIB-2.5	1973	The Genus Bacillus. not GLP, published Agricultural Research Service United States Department of Agriculture Washington D.C. Agriculture Handbook, 427, 1973, 36-253 BMF2000-54	N	-
Harwood, C. R.	AIIB-1.3; AIIB-2.1; AIIB-2.4; AIIB-2.6; AIIIB-4.4	1989	Introduction to the Biotechnology of Bacillus. not GLP, published Bacillus, The University of Newcastle upon Tyne, UK Plenum Press, 1989, 1-4 BMF2000-59	N	-
Priest, F. G.	AIIB-1.3; AIIB-2.1; AIIB-2.3; AIIB-2.4; AIIB-2.5; AIIB-2.6; AIIB-4.2	1993	Systematics and Ecology of Bacillus. not GLP, published in: Bacillus subtilis and other gram-positive bacteria American Society of Microbiology, Washington D.C. (ed.), 1993, 3-16 BMF2000-57	N	-
Priest, F.G.	AIIIB-1.4	1993	SYSTEMATICS AND ECOLOGY OF BACILLUS IN: BACILLUS SUBTILIS AND OTHER GRAM_POSITIVE BACTERIA. not GLP, published 1993, pp.3-16 PHY2000-718	N	-
Schlegel, H.G.	AIIB-1.3; AIIB-2.1; AIIB-2.4	1985	Allgemeine Mikrobiologie. not GLP, published Georg Thieme Verlag Stuttgart New York, 1985, 70-411 BMF2000-55	N	-

[2] Only notifier listed

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[3]
Schlegel, H.G.	AIIIB-1.4	1985	Die Zelle und Ihre Struktur; Das System der Prokapyoten; Unvollständige Oxydationen; Abbau von Naturstoffen. not GLP, published PHY2000-719	N	-
Slepecky, R. A.	AIIB-1.3; AIIB-2.4; AIIB-4.1	1992	What is a Bacillus?. not GLP, published in: Biology of Bacilli (Chapter 1) Doi, R. H. and Mc Gloughlin, M. (eds.) Application to Industry Buttenworth-Heinemann, Boston, 1992, 1-21 BMF2000-58	N	-

Codes of owner

QST: AgraQuest, Inc.

[3] Only notifier listed

A.2 Biological properties of the organism- Technical properties of the preparation (Annex IIB 2; Annex IIIB 2)

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[4]
Alabouvette, C. and Lemanceau, P.	AIIA-2.4; AIIB-2.1; AIIB-2.2	1998	Joint action of microbials for disease control (Article 8). not GLP, published Methods in Biotechnology, Vol. 5 Biopesticides: Use and Delivery, 1998, 117-135 BMF2000-78	N	-
Anonymous	AIIB-1.3; AIIB-2.5; AIIB-4.1	1997	Project Report SC 3817 - Bacterial Characterization ATCC. not GLP, unpublished BMF2000-56	Y	QST
Anonymous	AIIIA-2.2	2000	Minimum Explosive Concentration. K000726B.FMR not GLP, unpublished PHY2001-128	Y	QST
Anonymous	AIIIA-2.3	2001	Hintergrund Information zur Entflammbarkeit von Stäuben. not GLP, unpublished PHY2001-129	Y	QST
Anonymous	AIIIA-2.8.1; AIIIA-2.8.2; AIIIA-2.8.3; AIIIA-2.8.6	1999	Serenade WP Physical Property Analysis Summary. not GLP, unpublished PHY2001-65	Y	QST
Anonymous	AIIIB-2.1	2000	Physical/Chemical Properties. not GLP, unpublished PHY2000-720	N	QST
Asaka, O., Ano, T. and Shoda, M.	AIIB-2.1; AIIB-2.2; AIIB-2.4; AIIB-4.2	1996	Persistence of Bacillus subtilis RB14 and its derivative strains in soil with respect to the LPA-14 gene. not GLP, published J. of Fermentation and Bioengineering, Vol. 81, 1996, 1-6 BMF2000-92	N	-

[4] Only notifier listed

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[5]
Asaka, O., Tokuda, Y., Ano, T. and Shoda, M.	AIIB-2.7	1987	Plasmid instability in Bacillus subtilis during sporulation. not GLP, published Biosci. Biotech. Biochem. Vol. 19, No. 5, 1987, 639-647 BMF2000-155	N	-
Bar, J.G.	AIIB-2.8	1975	CHANGES IN THE EXTRACELLULAR ACCUMULATION OF ANTIBIOTICS DURING GROWTH AND SPORULATION OF BACILLUS SUBTILIS IN LIQUID CULTURE:. not GLP, published J. appl. Bact., Vol 39, 1975, 1-13 BMF2000-173	N	-
Bellet, E. M.	AIIB-1.4; AIIB-2.1	1998	Manufacturing and Analytical Data for QST 713 Technical. EPA Reg. No.: 69592-X GLP, unpublished BMF2000-66	Y	QST
Besson, F., Peypoux, F. and Michel, G.	AIIB-2.2; AIIB-2.8	1979	Antifungal activity upon Saccharomyces cerevisiae of Iturin A, Mycosubtilin, Bacillomycinl and of their derivates; inhibition of this antifungal activity by lipid antagonists. not GLP, published The J. of Antibiotics, Vol. XXXII, No. 8, 1979, 828-833 BMF2000-95	N	-
Besson, F., Peypoux, F, Michel, G. and Delcambe, L.	AIIB-2.8	1976	CHARACTERIZATION OF ITURIN A IN ANTIBIOTICS FROM VARIOUS STRAINS OF BACILLUS SUBTILIS:. not GLP, published The Journal of Antibiotics, Vol. XXIX, 10, 1976, 1043-1049 BMF2000-174	N	-

[5] Only notifier listed

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[6]
Besson, F., Peypoux, F., Quentin, M.J. and Michel, G.	AIIB-2.2	1984	Action of antifungal peptidolipids from Bacillus subtilis on the cell membrane of Saccharomyces cerevisiae. not GLP, published The J. of Antibiotics, Vol. XXXVII, No. 2, 1984, 172-177 BMF2000-96	N	-
Bland, J. M., Lax, A. R. and Klich, M. A.	AIIB-2.2	1990	Structure activity studies of the iturins. not GLP, published in: Peptides 1990 D. Giralt and D. Andreu (eds.) 1990, 426-427 BMF2000-97	N	-
Bland, J. M., Lax, A.R. and Klich, M.A.	AIIB-2.2; AIIB-2.8	1995	Iturin-A, a antifungal peptide produced by Bacillus subtilis. not GLP, published Pre. Plant Growth Regent Soc. Am. Vol. 22, 1995, 102-107 BMF2000-98	N	-
Boer de A. S. and Diderichsen, B.	AIIB-2.1; AIIB-2.5; AIIIB-4.3; AIIIB-4.4	1991	On the safety of Bacillus subtilis and B. amyloliquefaciens: A review. not GLP, published Appl. Microbiol. Biotechnol, Vol. 36, 1991, 1-4 BMF2000-91	N	-
Braun-Kiewnick, A. et al.	AIIB-2.2	1997	Induction of systemic resistance by antagonistic Bacillus sp. and the chemical inducer benzothiadiazole controls Cercospora leaf spot of sugar beet. not GLP, published Phytopathology, Vol. 87, No. 6 (Supplement), 1997, 10 BMF2000-99	N	-

[6] Only notifier listed

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[7]
Butt, T.M., Harris, J.G. and Powell, K.A.	AIIB-2.1; AIIB-2.2	1998	"Microbial biopesticides - the European scene" (Art. 3). not GLP, published in: Methods in Biotechnology, Vol. 5 Biopesticides, Use and Delivery, 1998, 23-44 BMF2000-85	N	-
Campbell, B., Cunningham-Hilbig, L.	AIIIB-2.6; AIIIB-4.4	2000	Serenade WP Tank Mix compability summary Report. not GLP, unpublished PHY2000-726	Y	QST
Campbell, B., Cunningham-Hilbig, L.	AIIIB-2.6; AIIIB-4.4; AIIIB-7.5	2000	Serenade WP Tank Mix compability interim Report. not GLP, unpublished PHY2000-725	Y	QST
Campbell, R.	AIIB-2.5	1989	Introduction to plant pathology and microbial ecology. not GLP, published in: Biological control of microbial plant pathogens, Cambridge University Press, Cambridge, Department of Botany University of Bristol (Chapter 1), 1989, 1-40 BMF2000-149	N	-
Campbell, R.	AIIB-2.2; AIIB-2.5; AIIB-4.2	1989	Biocontrol on leaf surfaces. not GLP, published in: Biological control of microbial plant pathogens Cambridge University Press, Cambridge, Department of Botany, University of Bristol Chapter 3, 1989, 66-94 BMF2000-100	N	-

[7] Only notifier listed

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[7]
Citernesi, A.S., Filippi, C., Bagnoli, G. and Giovannetti, M.	AIIB-2.1; AIIB-2.2; AIIB-2.3; AIIB-2.8	1994	Effects of the antimycotic iturin A2, secreted by Bacillus subtilis strain M51, on arbuscular mycorrhizal fungi. not GLP, published Microbiological research, Vol. 149, 1994, 241-246 BMF2000-71	N	-

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[8]
Cunningham Hilbig, Laura	AIIIA-2.6	2000	Liquid density test method using a pycnometer. FC4402 not GLP, unpublished PHY2001-131	Y	QST
Cunningham Hilbig, Laura	AIIIA-2.8.1	2000	Wet time method for powders & granules. FC4404 not GLP, unpublished PHY2001-132	Y	QST
Cunningham Hilbig, Laura Ryder Fox, Jennifer	AIIIA-2.8.3	2000	Suspensibility & Resuspensibility determination for dry products. FC4405 not GLP, unpublished PHY2001-133	Y	QST
Cunningham Hilbig, Laura Ryder Fox, Jennifer	AIIIA-2.8.5	2000	Wet sieve test method for powders & granules. FC4406 not GLP, unpublished PHY2001-134	Y	QST
Delcambe, L. et al.	AIIB-2.8	1977	STRUCTURE OF ITURIN AND ITURIN-LIKE SUBSTANCES:. not GLP, published Biochemical Society Transactions, Vol 5, 569th Meeting, Sussex, 1977, 1122-1124 BMF2000-175	N	-
Elsas v., J.D., Govaert, J.M. and Veen v. J.A.	AIIB-2.7	1987	Transfer of plasmid pFT30 between Bacilli in soil as influenced by bacterial population dynamics and soil conditions. not GLP, published Soil Biol. Biochem. Vol. 19, No. 5, 1987, 639-647 BMF2000-156	N	-

[8] Only notifier listed

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[8]
Elsasv. J.D., Dijkstra A.F., Govaert J.M. and Veen v. J.A.	AIIB-2.4; AIIB-4.2; AIIB-7.1	1986	Survival of Pseudomonas fluorescens and Bacillus subtilis introduced into two soils of different texture in field microplots. not GLP, published Federation of European Microbiological Societies, FEMS Microbiology Ecology 38, 1986, 151-160 BMF2000-146	N	-

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[9]
EPA	AIIB-2.1; AIIB-2.5; AIIB-2.8; AIIB-2.9; AIIB-3.7; AIIIB-4.3; AIIIB-4.4	1997	Final Decision Document, TSCA Section 5 (H) (4) Exemption for Bacillus subtilis. not GLP, published EPA, 1997 BMF2000-93	N	-
Eshita, S.M. et al.	AIIB-2.1; AIIB-2.2	1995	Bacillomycin Lc, a new antibiotic of the iturin group: isolation, structures and antifungal activities of the congeners. not GLP, published J. of Antibiotics, Vol. 48, No. 11, 1995, 1240-1247 BMF2000-84	N	-
Feignier, C., Besson, F. and Michel, G.	AIIB-2.8	1995	STUDIES ON LIPOPEPTIDE BIOSYNTHESIS BY BACILLUS SUBTILIS: ISOLATION AND CHARACTERIZATION OF ITURIN-; SURFACTIN+ MUTANTS:. not GLP, published FEMS Microbiology Letters 127, 1995, 11-15 BMF2000-176	N	-
Ferrari, E. and Hoch, J.A.	AIIB-2.7	1989	Genetics. not GLP, published in: Bacillus, Colin R. Harwood (ed), The University of Newcastle upon Tyne Newcastle upon Tyne, UK, Plenum Press, 1989, 57-72 BMF2000-157	N	-
Fossum, K., Herikstad, H., Binde, M. and Pettersen K.-E.	AIIB-2.5; AIIB-2.9	1986	Isolations of Bacillus subtilis in connection with Bovine Mastitis. not GLP, published Nordisk Veterinärmedicine, Vol. 38, 1986, 233-236 BMF2000-151	N	-

[9] Only notifier listed

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[10]
Gingras, B. A.	AIIIB-2.2; AIIIB-4.4; AIIIB-5.1	1999	Storage Stability of Qst 713 Strain of Dried Bacillus subtilis with-Residual Fermentation media Identified as Qst 713 WP. Project No. L08726 SN9 GLP, unpublished PHY2000-721	Y	QST
Gingras, B. A.	AIIIB-2.6	2000	Serenade WP Tank Mix compability interim Report. not GLP, unpublished PHY2000-727	Y	QST
Glick, B.R. et al.	AIIB-2.3; AIIB-2.5	1999	Overview of plant growth-promoting bacteria. not GLP, published in: Biochemical and Genetic Mechanisms used by plant growth promoting bacteria, (Chapter 1) Imperial College Press, Department of Biology, University of Waterloo, Ontario, Canada, 1999, 1-13 BMF2000-89	N	-
Glick, B.R., Patten, C.L., Holguin, G. and Penrose, D.M.	AIIB-2.7	1999	Deliberate environmental release of bacteria. not GLP, published in: Biochemical and Genetic Mechanisms used by plant growth promoting bacteria, Imperial College Press, Department of Biology, University of Waterloo, Ontario, Canada (Chapter 8), 1999, 249-267 BMF2000-158	N	-
Gordon, R.E., Haynes, W.C. and Pang, C.H.-N.	AIIB-1.3; AIIB-2.1; AIIB-2.5	1973	The Genus Bacillus. not GLP, published Agricultural Research Service United States Department of Agriculture Washington D.C. Agriculture Handbook, 427, 1973, 36-253 BMF2000-54	N	-

[10] Only notifier listed

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[11]
Graham, J.B. and Istock, C.A.	AIIB-2.7	1979	Gene exchange and natural selection cause Bacillus subtilis to evolve in soil culture. not GLP, published Science Vol. 204, 1979, 637-639 BMF2000-159	N	-
Gueldner, R.C.	AIIB-2.2	1988	Isolation and identification of iturins as anti-fungal peptides in biological controls of peach brown rot with Bacillus subtilis. not GLP, published J. Agric. Food Chem., Vol. 36, 1988, 366-369 BMF2000-101	N	-
Harwood, C. R.	AIIB-1.3; AIIB-2.1; AIIB-2.4; AIIB-2.6; AIIIB-4.4	1989	Introduction to the Biotechnology of Bacillus. not GLP, published Bacillus, The University of Newcastle upon Tyne, UK Plenum Press, 1989, 1-4 BMF2000-59	N	-
Harwood, C.R. (ed.)	AIIB-2.9	1989	BACILLUS. not GLP, published The University of Newcastle upon Tyne, UK, Plenum Press (UPDATED LINKAGE MAP OF B: SUBTILIS in Annex X, 1989, 363-406 BMF2000-179	N	-
Hemilä, H., Glode, L.M. and Palva, I.	AIIB-2.7	1989	Production of diphteria toxin CRM228 in B. subtilis. not GLP, published Fed. Eur. Micrbiol. Soc. Lett., Vol. 65, 1989, 193-198 BMF2000-160	N	-
Hiraoka, H., Asaka, O., Ano, T. and Shoda, M.	AIIB-2.1; AIIB-2.2	1992	Characterization of Bacillus subtilis RB14, coproducer of peptide antibiotics iturin A and surfactin. not GLP, published J. Gen. Appl. Microbiol., Vol. 38, 1992, 635-640 BMF2000-82	N	-

[11] Only notifier listed

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[12]
Ihde, D.C. and Armstrong D.	AIIB-2.5; AIIB-2.9	1973	Clinical spectrum of infection due to bacillus species. not GLP, published Amer. J. Med., Vol. 55, 1973, 839-845 BMF2000-152	N	-
Jacobsen, B. J. and Zidack, N.K.	AIIB-2.2	2000	Induction of disease resistance mechanismus in by Bacillus subtilis strain QST713 and disease control of Erwinia caratovora subsp. betavas-culorum in sugar beet. not GLP, unpublished BMF2000-94	Y	QST
Kararah M.A. et al.	AIIB-2.3; AIIB-2.4; AIIB-2.5	1985	Pathophysiology in garlic gloves inoculated with Bacillus subtilis, Bacillus pumilis and Erwinia carotovora. not GLP, published Egyptian J. Phytopathology Vol. 17 (2), 1985, 131-140 BMF2000-145	N	-
Katz, E. and Demain, A.L.	AIIB-2.8	1977	THE PEPTIDE ANTIBIOTICS OF BACIL-LUS: CHEMISTRY; BIOGENESIS; AND POSSIBLE FUNCTIONS. not GLP, published Bacteriological Reviews, 1977, 449-474 BMF2000-177	N	-
Kilian, M. et al.	AIIB-2.1; AIIB-2.2; AIIB-2.3; AIIB-2.4	1998	FZB24 Bacillus subtilis - Ein Pflanzenstär-kungsmittel für den kartoffelanbau. not GLP, published Mitt. a. d. Biol. Bundesanst. H. 357, No. 509a, 1998, 362 BMF2000-86	N	-
Kilian, M., Junge, H. und Krieg, U.	AIIB-2.5	1998	Einfluß von Umweltfaktoren auf die ertrags-steigernde Wirkung von FZB24 Bacillus subti-lis bei Kartoffeln. not GLP, published Mitt. a. d. Biol. Bundesanst., 509, 357, 1998, 361 BMF2000-153	N	-

[12] Only notifier listed

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[13]
Klich M.A., Lax A.R. and Bland J.M.	AIIB-2.2; AIIB-2.8	1991	Inhibition of some mycotoxigenic fungi by iturin A, a peptidolipid produced by Bacillus subtilis. not GLP, published Mycopathologia, Vol. 116, 1991, 77-80 BMF2000-140	N	-
Klier, A., Bourgouin, C. and Rapoport, G.	AIIB-2.7	1983	Mating between Bacillus subtilis and Bacillus thuringiensis and transfer of cloned crystal genes. not GLP, published Mol. Gen. Gent., Vol. 191, 1983, 257-262 BMF2000-161	N	-
Kloepper, J.W., Lifshitz, R., Zablotowicz, R.M.	AIIB-2.1	1989	Free-Living bacterial inocula for enhancing crop productivity. not GLP, published Tibtech, Vol. 7, 1989, 39-44 BMF2000-88	N	-
Latoud, C., Peypoux, F. and Michel, G	AIIB-2.8	1987	ACTION OF ITURIN A, AN ANTIFUNGAL ANTIBIOTIC FROM BACILLUS SUBTILIS; ON THE YEAST SACCHAROMYCES CEREVISIAE: MODIFICATIONS OF MEMBRANE PERMEABILITY AND LIPID COMPOSITION. not GLP, published The Journal of Antibiotics, Vol. XL, 11, 1987, 1588-1595 BMF2000-178	N	-
Leifert, C., Chidburee S., hampson S., Workman S., Sigee D., Epton H.A.S. and Harbour A.	AIIB-2.2; AIIB-2.5	1995	Antibiotic production and biocontrol activity by Bacillus subtilis CL27 and Bacillus pumilus CL45. not GLP, published Journal of Applied Bacteriology, Vol. 78, 1995, 97-108 BMF2000-141	N	-

[13] Only notifier listed

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[13]
Leonhardt, H. and Alonso, J.C.	AIIB-2.7	1991	Parameters affecting plasmid stability in Bacillus subtilis. not GLP, published Gene, Vol. 103, 1991, 107-111 BMF2000-162	N	-

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[14]
Loeffler W., Tschen J.S.-M., Vanittanakom N., Kugler M., Knorrp E., Hsieh T.-F. and Wu T.-g.	AIIB-2.2; AIIB-2.5	1986	Antifungal effects of Bacilysin and Fengmycin from Bacillus subtilis F-29-3 A comparison with activities of other Bacillus antibiotics. not GLP, published J. Phytopathology, Vol. 115 (3), 1986, 204-213 BMF2000-142	N	-
Loper J.E. and Lindow S.E.	AIIB-2.2	1993	Roles of competition and antibiosis in suppression of plant diseases by bacterial biological control agents. not GLP, published Pest management: Biologically Based Technologies, 1993, 144-155 BMF2000-143	N	-
Marten, P., Brückner, S. and Lüth, P.	AIIB-2.1; AIIB-2.2; AIIB-2.3	1998	Wachstumsförderung und Biologische Kontrolle durch Bacillus subtilis Stamm B2g. not GLP, published Mitt. a.d. Biol. Bundesanst. H 357, 1998, 357 BMF2000-87	N	-
Matsuno, C.D., Hitomi, T., Ano, T. and Shoda M.	AIIB-2.1; AIIB-2.7; AIIB-2.8; AIIB-2.9	1992	Transformation of Bacillus subtilis, antifungal-antibiotic iturin producers with isolated antibiotic resistance plasmids. not GLP, published J. Gen. Appl. Microbiol., Vol. 38, 1992, 13-21 BMF2000-77	N	-
McKeen, C.D., Reilly, C.C. and Pusey, P.L.	AIIB-2.1; AIIB-2.2	1986	Production and partial characterization of antifungal substances antagonistic to Monilia fructicola from Bacillus subtilis. not GLP, published Phytopathology, Vol. 76, No. 2, 1986, 136-138 BMF2000-80	N	-

[14] Only notifier listed

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[14]
Mountain, A.	AIIB-2.7	1989	Gene expression systems for Bacillus subtilis. not GLP, published in: Bacillus, Colin R. Harwood (ed) The University of Newcastle upon Tyne Newcastle Tyne, UK, Plenum Press (Chapter 5), 1989, 73-114 BMF2000-163	N	-

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[15]
O`Donnel, A.G., Norris J.R., Berkeley R.C.W., Claus D., Kaneko T., Logan N.A. and Nozaki R.	AIIB-2.1	1980	Characterization of bacillus subtilis, Bacillus pumilus, Bacillus licheniformis, and Bacillus amyloliquefaciens by Pyrolysis Gas-Liquid Chromatography, Deoxyribonucleic Acid-Deoxyribonucleic Acid Hybridization, bio-chemical Tests, and API Systems. not GLP, published International J. of systematic Bacteriology Vol.30, No. 2, 1980, 448-459 BMF2000-139	N	-
Ohno, A., Ano, T. and Shoda, M.	AIIB-2.1	1995	Effect of temperature on production of lipo-peptide antibiotics, iturin A and surfactin by a dual producer, Bacillus subtilis RB14, in solid-state fermentation. not GLP, published J. of Fermentation and Bioengineering, Vol. 80, No. 5, 1995, 517-519 BMF2000-73	N	-
Ohno, A., Ano, T. and Shoda, M.	AIIB-2.1; AIIB-2.5	1993	Effect of temperature change and aeration on the production of the antifungal peptide antibi-otic iturin by Bacillus subtilis NB22 in liquid cultivation. not GLP, published J. of Fermentation and Bioengineering, Vol. 75, No. 1, 1993, 463-465 BMF2000-72	N	-
Ohno, A., Ano, T. and Shoda, M.	AIIB-2.7	1995	Production of a lipopeptide antibiotic surfactin, by recombinant Bacillus subtilis in solid state fermentation. not GLP, published Biotechnology and Bioengineering, Vol. 47 (2), 1995, 209-214 BMF2000-164	N	-

[15] Only notifier listed

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[15]
Phae, C.-G. and Shoda, M.	AIIB-2.1	1991	Investigation of optimal conditions for foam separation of iturin, an antifungal peptide produced by Bacillus subtilis. not GLP, published J. of fermentation and Bioengineering, Vol. 71, No. 2, 1991, 118-121 BMF2000-75	N	-

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[16]
Phae, C.-G., Sasaki, M., Shoda, M. and Kubota, H.	AIIB-2.1; AIIB-2.2; AIIB-2.5; AIIB-2.9	1990	Characteristics of Bacillus subtilis isolated from composts suppressing phytopathogenic microorganisms. not GLP, published Soil Sci. Plant Nutr., Vol. 36(4), 1990, 575-586 BMF2000-74	N	-
Priest, F. G.	AIIB-1.3; AIIB-2.1; AIIB-2.3; AIIB-2.4; AIIB-2.5; AIIB-2.6; AIIB-4.2	1993	Systematics and Ecology of Bacillus. not GLP, published in: Bacillus subtilis and other gram-positive bacteria American Society of Microbiology, Washington D.C. (ed.), 1993, 3-16 BMF2000-57	N	-
Priest, F.G.	AIIB-2.1; AIIB-2.5	1989	Isolation and identification of aerobic endospore-forming Bacteria. not GLP, published in: Bacillus, Chapter 3 Colin R. Harwood (ed.) The University of Newcastle upon Tyne, UK Plenum Press 1989, 27-56 BMF2000-90	N	-
Priest, F.G.	AIIB-2.1; AIIB-2.5; AIIB-2.8	1989	Products and Applications. not GLP, published in: Bacillus, Colin R. Harwood (ed.) The University of Newcastle upon Tyne, UK, Plenum Press, Chapter 11, 1989, 293-320 BMF2000-69	N	-
Pusey, P.L. and Wilson, C.L.	AIIB-2.1	1984	Postharvest biological control of stone fruit brown rot by Bacillus subtilis. not GLP, published Plant Disease, Vol. 68, No. 9, 1984, 753-756 BMF2000-79	N	-

[16] Only notifier listed

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[17]
Pusey, P.L. et al.	AIIB-2.1	1988	Pilot tests for commercial production and application of Bacillus subtilis (B-3) for postharvest control of peach brown rot. not GLP, published Plant Disease, Vol. 72, No. 7, 1988, 622-626 BMF2000-76	N	-
Ryder Fox, Jennifer	AIIIA-2.6	2000	Bulk density Test Method for Powders & Granules. FC4401 not GLP, unpublished PHY2001-130	Y	QST
Saris, P., Taira, S., Airaksinen, U., Palva A., Sarvas, M., Palva I. and Runeberg-Nyman, K.	AIIB-2.7; AIIB-2.9	1990	Production and secretion of pertussis toxin subunits in Bacillus subtilis. not GLP, published FEMS Microbiology Letters, Vol. 68, 1990, 143-148 BMF2000-166	N	-
Saris, P.E.J., Airaksinen, U., Nurmiharju, S., Runeberg-Nyman, K. and Palva I.	AIIB-2.7; AIIB-2.9	1990	Expression of Bordetella pertussis toxin subunits in Bacillus subtilis. not GLP, published Biotechnology Letters, Vol. 12 (12), 1990, 873-878 BMF2000-165	N	-
Schlegel, H.G.	AIIB-1.3; AIIB-2.1; AIIB-2.4	1985	Allgemeine Mikrobiologie. not GLP, published Georg Thieme Verlag Stuttgart New York, 1985, 70-411 BMF2000-55	N	-
Schmiedeknecht, G. et al.	AIIB-2.1; AIIB-2.3	1998	Anwendungsmöglichkeiten von Bacillus subtilis für den Biologischen Pflanzenschutz. not GLP, published Mitt. a.d. Biol. Bundesanst. H. 357, 1998, 354 BMF2000-83	N	-

[17] Only notifier listed

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[18]
Sholberg, P.L., Marchi, A. and Bechard, J.	AIIB-2.1; AIIB-2.2; AIIB-2.5	1995	Biocontrol of postharvest diseases of apple using Bacillus spp. isolated from stored apples. not GLP, published Can. J. Microbiol. 41, 1995, 247-252 BMF2000-81	N	-
Slepecky, R. A.	AIIB-1.3; AIIB-2.4; AIIB-4.1	1992	What is a Bacillus?. not GLP, published in: Biology of Bacilli (Chapter 1) Doi, R. H. and Mc Gloughlin, M. (eds.) Application to Industry Buttenworth-Heinemann, Boston, 1992, 1-21 BMF2000-58	N	-
Sneath, P.H.A.	AIIB-2.1; AIIB-2.5	1986	Endospore-forming gram-positive rods and cocci. not GLP, published in: Bergey`s Manual of Systematic Bacteriology Claus, D. and Berkely, R.C.W. (eds.) The Williams & Wilkens Co., Baltimore, Vol.2, 1986, 1104-1139 BMF2000-67	N	-
Stanghellini, M.E. and rasmussen, S.L.	AIIB-2.3	1989	Two new diseases of Salicornia sp. caused by Bacillus subtilis and Macrophomina phaseolina. not GLP, published Phytopathology Vol. 79 (8), 1989, 912 BMF2000-150	N	-
Taira, S., Jalonen, E., Paton, J.C., Sarvas M. and Runeberg-Nyman, K.	AIIB-2.7; AIIB-2.9	1989	Production of pneumolysin, a pneumococcal toxin in Bacillus subtilis. not GLP, published Gene, Vol. 77, 1989, 211-218 BMF2000-167	N	-
Thimon L., Maget-Dana R. and Michel G.	AIIB-2.2; AIIB-2.8	1992	Surface active properties of antifungal lipopeptides produced by Bacilluis subtilis. not GLP, published JAOCS, Vol. 69 (1), 1992, 92-93 BMF2000-144	N	-

[18] Only notifier listed

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[19]
Tokuda, Y., Ano, T. and Makoto, S.	AIIB-2.7; AIIB-2.9	1993	Characterictics of plasmid stability in Bacillus subtilis NB22, an antifungal antibiotic iturin producer. not GLP, published J. of Fermentation and Bioengineering, Vol. 75 (4), 1993, 319-321 BMF2000-168	N	-
Walter, D.	AIIIB-2.6	2000	Surface Tension of QST 713 WP. Study code: 99430/01-PCST GLP, unpublished PHY2000-723	N	QST
Walter, Dieter	AIIIB-2.1; AIIIB-2.2; AIIIB-2.4.1; AIIIB-2.4.2; AIIIB-2.4.4; AIIIB-2.4.5	2000	Physico-chemical Properties of the Formulation QST 713 WP after Accelerated Storage at 40°C for 8 Weeks. 99430/01-PCAS GLP, unpublished PHY2000-722	Y	QST
Yuan, C. and Heins, S.	AIIB-2.5	2000	Longevity study of Serenade (QST 713) on pepper leaf surface in greenhouse conditions. AQ0032 not GLP, unpublished BMF2000-148	Y	QST
Zimmer, J., Issoufou I., Schmiedeknecht, G. und Bochow, H.	AIIB-2.5	1998	Populationsdynamik, Phytoeffektivität und antagonistische Wirksamkeit von Bacillus subtilis als Nutzbakterium. not GLP, published Mitt. a.d. Biol. Bundesanst., 357, 1998, 351 BMF2000-154	N	-
Zimmermann, S.B. et al.	AIIB-2.1; AIIB-2.8	1987	Difficidin and Oxydifficidin: Novel broad spectrum antibacterial antibiotics produced by Bacillus subtilis. not GLP, published J. Antibiotics, Vol. 40 (12), 1987, 1677-1681 BMF2000-70	N	-

Codes of owner
QST: AgraQuest, Inc.

[19] Only notifier listed

A.3 Further information (Annex IIA 3; Annex IIIA 3, 4 and 6)

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[20]
Anonymous	AIIB-3.7	1998	BPPD COMPANY FEDERAL REGISTER DOCUMENT SUBMISSION TEMPLATE (12/1/98). not GLP, unpublished BMF2000-182	Y	QST
Anonymous	AIIIB-3.9; AIIIB-4.4	2000	Serenade WP Instrucions for Use. not GLP, unpublished PHY2001-71	N	QST
Anonymous	AIIIB-4.4; AIIIB-12.3	2000	Safety Data Sheet: Serenade WP. not GLP, unpublished PHY2001-69	N	QST
Boer de A. S. and Diderich-sen, B.	AIIB-2.1; AIIB-2.5; AIIIB-4.3; AIIIB-4.4	1991	On the safety of Bacillus subtilis and B. amy-loliquefaciens: A review. not GLP, published Appl. Microbiol. Biotechnol, Vol. 36, 1991, 1-4 BMF2000-91	N	-
Campbell, B., Cunningham-Hilbig, L.	AIIIB-2.6; AIIIB-4.4	2000	Serenade WP Tank Mix compability summary Report. not GLP, unpublished PHY2000-726	Y	QST
Campbell, B., Cunningham-Hilbig, L.	AIIIB-2.6; AIIIB-4.4; AIIIB-7.5	2000	Serenade WP Tank Mix compability interim Report. not GLP, unpublished PHY2000-725	Y	QST
Duncan, R. A. and Leung, P.	AIIB-3.7	1999	PRODUCT REGISTRATION RECOMMEN-DATION SHEET. not GLP, unpublished BMF2000-183	Y	QST

[20] Only notifier listed

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[21]
EPA	AIIB-2.1; AIIB-2.5; AIIB-2.8; AIIB-2.9; AIIB-3.7; AIIIB-4.3; AIIIB-4.4	1997	Final Decision Document, TSCA Section 5 (H) (4) Exemption for Bacillus subtilis. not GLP, published EPA, 1997 BMF2000-93	N	-
Frommer, W. et al.	AIIB-3.7; AIIIB-4.3	1989	SAFE BIOTECHNOLOGY III. SAFETY PRECAUTIONS FOR HANDLING MICRO-ORGANISMS OF DIFFERENT CLASSES. not GLP, published Appl. Microbiol. Biotechnol., Vol. 30, 1989, 541-552 BMF2000-184	N	-
Gingras, B. A.	AIIIB-2.2; AIIIB-4.4; AIIIB-5.1	1999	Storage Stability of Qst 713 Strain of Dried Bacillus subtilis with-Residual Fermentation media Identified as Qst 713 WP. Project No. L08726 SN9 GLP, unpublished PHY2000-721	Y	QST
Harwood, C. R.	AIIB-1.3; AIIB-2.1; AIIB-2.4; AIIB-2.6; AIIIB-4.4	1989	Introduction to the Biotechnology of Bacillus. not GLP, published Bacillus, The University of Newcastle upon Tyne, UK Plenum Press, 1989, 1-4 BMF2000-59	N	-
Li, H. and Leifert, C	AIIB-3.5	1994	DEVELOPMENT OF RESISTANCE IN BOTRYOTINIA FUCKELIANA (DE BARY) WHETZEL AGAINST THE BIOLOGICAL CONTROL AGENT BACILLUS SUBTILIS CL27. not GLP, published Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz, Vol. 101, 1994, 414-418 BMF2000-180	N	-

[21] Only notifier listed

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[22]
Roush, R.T.	AIIB-3.5	1998	STRATEGIES FOR RESISTANCE MANAGEMENT (Art. 30). not GLP, published Methods in Biotechnology, Vol. 5: Biopesticides: Uses and Delivery, 1998, 575-593 BMF2000-181	N	-

Codes of owner

QST: AgraQuest, Inc.

[22] Only notifier listed

A.4 Classification and labelling

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[23]
Anonymous	AIIIB-4.4; AIIIB-12.3	2000	Safety Data Sheet: Serenade WP. not GLP, unpublished PHY2001-69	N	QST

Codes of owner

QST: AgraQuest, Inc.

[23] Only notifier listed

A.5 Methods of analysis (Annex IIB 4; Annex IIIB 5)

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[24]
Anonymous	AIIB-1.3; AIIB-2.5; AIIB-4.1	1997	Project Report SC 3817 - Bacterial Characterization ATCC. not GLP, unpublished BMF2000-56	Y	QST
Asaka, O., Ano, T. and Shoda, M.	AIIB-2.1; AIIB-2.2; AIIB-2.4; AIIB-4.2	1996	Persistence of Bacillus subtilis RB14 and its derivative strains in soil with respect to the LPA-14 gene. not GLP, published J. of Fermentation and Bioengineering, Vol. 81, 1996, 1-6 BMF2000-92	N	-
Campbell, R.	AIIB-2.2; AIIB-2.5; AIIB-4.2	1989	Biocontrol on leaf surfaces. not GLP, published in: Biological control of microbial plant pathogens Cambridge University Press, Cambridge, Department of Botany, University of Bristol Chapter 3, 1989, 66-94 BMF2000-100	N	-
Collins, C.H., Lyne, P.M. and Grange, J.M.	AIIB-4.1	1989	MICROBIOLOGICAL METHODS. not GLP, published Buttenworths, London (4095), Chapter 7: Identification Methods, 1989, 97-114 BMF2000-187	N	-
Elsasv. J.D., Dijkstra A.F., Govaert J.M. and Veen v. J.A.	AIIB-2.4; AIIB-4.2; AIIB-7.1	1986	Survival of Pseudomonas fluorescens and Bacillus subtilis introduced into two soils of different texture in field microplots. not GLP, published Federation of European Microbiological Societies, FEMS Microbiology Ecology 38, 1986, 151-160 BMF2000-146	N	-

[24] Only notifier listed

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[25]
Gingras, B. A.	AIIIB-2.2; AIIIB-4.4; AIIIB-5.1	1999	Storage Stability of Qst 713 Strain of Dried Bacillus subtilis with-Residual Fermentation media Identified as Qst 713 WP. Project No. L08726 SN9 GLP, unpublished PHY2000-721	Y	QST
Gingras, B.A.	AIIB-4.1	1999	STORAGE STABILITY OF TECHNICAL QST 713 (FINAL REPORT). L08726 SN2 GLP, unpublished BMF2000-186	Y	QST
Heins, S.D.	AIIB-4.1	1999	METHODS FOR DETERMINATION OF AEROBIC COLONY FORMING UNITS UTILIZING SPIRAL PLATER (DSOP#: FM4201). GLP, unpublished BMF2000-185	Y	QST
Priest, F. G.	AIIB-1.3; AIIB-2.1; AIIB-2.3; AIIB-2.4; AIIB-2.5; AIIB-2.6; AIIB-4.2	1993	Systematics and Ecology of Bacillus. not GLP, published in: Bacillus subtilis and other gram-positive bacteria American Society of Microbiology, Washing-ton D.C. (ed.), 1993, 3-16 BMF2000-57	N	-
Priest, F.G. et al.	AIIB-4.1	1988	A NUMERICAL CLASSIFICATION OF THE GENUS BACILLUS. not GLP, published J. Gen. Microbiol., Vol 134, 1988, 1847-1882 BMF2000-188	N	-

[25] Only notifier listed

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[26]
Slepecky, R. A.	AIIB-1.3; AIIB-2.4; AIIB-4.1	1992	What is a Bacillus?. not GLP, published in: Biology of Bacilli (Chapter 1) Doi, R. H. and Mc Gloughlin, M. (eds.) Application to Industry Buttenworth-Heinemann, Boston, 1992, 1-21 BMF2000-58	N	-

Codes of owner

QST: AgraQuest, Inc.

[26] Only notifier listed

A.6 Toxicity, pathogenicity and infectivity (Annex IIB 5; Annex IIIB 7)

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[27]
Anonym	AIIB-5.1.3; AIIB-5.4.1; AIIB-5.4.2	1997	Final decision document: TSCA section 5(H)(4) exemption for bacillus subtilis. not GLP, published EPA, 1997 TOX2000-1216	N	-
Bellet, E.	AIIIB-7.1.1	1998	Acute oral exposure toxicity study in rats with QST 713 WP. 0402XA54.001 GLP, unpublished TOX2000-1199	Y	QST
Bellet, E.	AIIIB-7.1.3	1998	Acute exposure dermal toxicity in rabbits with QST 713 WP. 0422XA54.001 GLP, unpublished TOX2000-1201	Y	QST
Bellet, E.	AIIIB-7.2.1	1998	Primary dermal irritation in rabbits with QST 713 WP. 0420XA54.003 GLP, unpublished TOX2000-1202	Y	QST
Bellet, E.	AIIIB-7.2.2	1998	Primary eye irritation in rabbits with QST 713 WP. 0421XA54.003 GLP, unpublished TOX2000-1203	Y	QST
Bellet, E.	AIIB-5.1.1.7; AIIIB-7.2.3	1998	Delayed contact hypersensitivity in guinea pigs with QST 713 WP (Buehler Method). 0424XA54.001 GLP, unpublished TOX2000-1204	Y	QST
Campbell, B., Cunningham-Hilbig, L.	AIIIB-2.6; AIIIB-4.4; AIIIB-7.5	2000	Serenade WP Tank Mix compability interim Report. not GLP, unpublished PHY2000-725	Y	QST

[27] Only notifier listed

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[28]
Douds, G.A.	AIIIB-7.1.2	1998	An acute whole-body inhalation toxicity study in rats with QST 713 WP. 3474.1 GLP, unpublished TOX2000-1200	Y	QST
Findlay, J.	AIIB-5.1.1.3; AIIB-5.1.1.6	1998	Acute dermal toxicity/pathology study of QST 713 in rabbits. L08726SN7 GLP, unpublished TOX2000-1218	Y	QST
Frommer, W. et al.	AIIB-5.1	1989	Safe biotechology. III. Safety precautions for handling microorganisms of different risk classes. not GLP, published Appl. Microbiol. Biotechnol., 30, 1989, 541-552 TOX2000-1215	N	-
Gingras, B.A.	AIIB-5.1.1.1	1998	Sensitivity of detection of bacillus subtilis strain QST 713 for toxicity/pathogenicity testing in rats. L08726 SN3 GLP, unpublished TOX2000-1207	Y	QST
Harrington, K.	AIIB-5.1.1.4	1998	Toxicity/pathogenicity testing of QST 713 following acute intatracheal challenge in rats. L08726 SN6 GLP, unpublished TOX2000-1208	Y	QST
Harrington, K.A.	AIIB-5.1.1.1	1998	Toxicity/pathogenicity testing of QST 713 following acute oral challenge in rats. L08726 SN4 GLP, unpublished TOX2000-1206	Y	QST

[28] Only notifier listed

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[29]
Harrington, K.A.	AIIB-5.1.2.2	1998	Toxicity/pathogenicity testing of QST 713 following acute intravenous challenge in rats. L08726SN5 GLP, unpublished TOX2000-1219	Y	QST
Harwood, C.R.	AIIB-5.1.2	2000	Introduction to the biotechnology of bacillus. not GLP, published Bacillus, The university of Newcastle upon tyne, UK, Plenum Press, 1-4 TOX2000-1214	N	-
Ihde, D.C. and Armstrong, D.	AIIB-5.1; AIIB-5.4.3	1973	Clinical spectrum of infection due to bacillus species. not GLP, published The American Journal of Medicine., 55, 1973, 839-845 TOX2000-1217	N	-
Mallory, V.T.	AIIB-5.1.1.6	1998	Primary eye irritation in rabbits with QST 713 TP. 0421XA054.004 GLP, unpublished TOX2000-1211	Y	QST
Mallory, V.T.	AIIB-5.1.1.6	1998	Primary dermal irritation in rabbits with QST 713 TP. 0420XA054.004 GLP, unpublished TOX2000-1210	Y	QST
Sietske de Boer, A. and Diderichsen, B.	AIIB-5.1; AIIB-5.4.1; AIIB-5.4.3; AIIB-5.4.4	1991	On the safety of bacillus subtilis and b. amyloliquefaciens: a review. not GLP, published Appl. Microbiol. Biotechnol., 36, 1991, 1-4 TOX2000-1212	N	-
Taira, S., Jalonen, E., Paton, J.C., Sarvas, M. and Runeberg-Nyman, K.	AIIB-5.2.3	1989	Production of pneumolysin, a pneumococcal toxin, in bacillus subtilis. not GLP, published Gene, 77, 1989, 211-218 TOX2000-1213	N	-

[29] Only notifier listed

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[30]
Thobor, C.	AIIB-5.5; AIIIB-7.6	2000	Bacillus subtilis (150g/kg technical powder) & Serenade tm WP (AI: 100g/kg, formulation: wettable powder) - Summary of mammalian toxicity, pathogenicity and infectivity, exposure risk assessments and overall evaluation. AB981202-EU09/01 not GLP, published TOX2000-1205	Y	-

Codes of owner

QST: AgraQuest, Inc.

[30] Only notifier listed

A.7 Residues in or on treated products, food and feed (Annex IIB 6; Annex IIIB 8)

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[31]
Boer de A. S.; Diderichsen, B.	AIIB-6.2	1991	On the safety of Bacillus subtilis and B. amyloliquefaciens: a review. not GLP, published Appl. Microbiol. Biotechnol, Vol. 36, 1991, pp.1-4 RIP2000-2005	N	-
Campbell, R.	AIIB-6.1	1989	Biocontrol on Leaf Surfaces. not GLP, published Cambridge University Press, Cambridge, Department of Botany, Universität of Bristof, 1989, pp. 66-94 RIP2000-2000	Y	-
EPA	AIIB-6.1	1997	Final Decision Document, TSCA Section 5 (H) (4) Exemption for Bacillus Subtilis. not GLP, published RIP2000-2001	N	-
Priest, F.G.	AIIB-6.1	1993	Systematics and Ecology of Bacillus in: Bacillus subtilis and other Gram-Positive Bacteria. not GLP, unpublished RIP2000-2006	Y	QST
Priest, F.G.	AIIB-6.1	1993	Systematics and Ecology of Bacillus in: Bacillus subtilis and other Gram-Positive Bacteria. not GLP, unpublished RIP2000-2002	Y	QST
Sholberg, P.L.; Marchi, A.; Bechard, J.	AIIB-6.1; AIIB-6.2	1995	Biocontrol of postharvest diseases of apple using Bacillus spp. isolated from stored apples. not GLP, published Can. J. Microbiol. 41, 1995, pp.247-25 RIP2000-2004	N	-

[31] Only notifier listed

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[32]
Yuan, C.; Heins, S.	AIIB-6.1	2000	Longevity Study of Serenade (QST713) on Pepper Leaf Surface in Greenhouse Conditions. Report No. AQ0032 not GLP, unpublished RIP2000-2003	Y	QST
Yuan, C.; Heins, S.	AIIB-6.1; AIIB-6.2	2000	Longevity Study of Serenade (QST713) on Pepper Leaf Surface in Greenhouse Conditions. Report No. AQ0032 not GLP, unpublished RIP2000-1998	Y	QST

Codes of owner

QST: AgraQuest, Inc.

[32] Only notifier listed

A.8 Fate and behaviour in the environment (Annex IIB 7; Annex IIIB 9)

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[33]
Anonymous	AIIB-7	1997	Final Decision Document: TSCA Section 5(H)(4) Exemption for Bacillus subtilis. 3176 not GLP, unpublished BOD2000-1366	N	QST
Asaka, O., Ano, T. and Shoda, M.	AIIB-7	1996	Persistence of Bacillus subtilis RB14 and Its Derivative Strains in Soil with Respect to the lpa-14 Gene. not GLP, published Journal of Fermantation and Bioengineering, 81, 1, 1996, 1-6 BOD2000-1364	N	-
Campbell, R.	AIIB-7	1989	Introduction to plant pathology and microbial ecology. not GLP, published Cambridge Univertity Press, 1989, 1-40 BOD2000-1372	N	-
Elsasv. J.D., Dijkstra A.F., Govaert J.M. and Veen v. J.A.	AIIB-2.4; AIIB-4.2; AIIB-7.1	1986	Survival of Pseudomonas fluorescens and Bacillus subtilis introduced into two soils of different texture in field microplots. not GLP, published Federation of European Microbiological Societies, FEMS Microbiology Ecology 38, 1986, 151-160 BMF2000-146	N	-
Glick et al.	AIIB-7	1999	Deliberate Environmental Release of Bacteria. not GLP, published Mechanisms Used by Plant-Growth Promoting Bacteria, 1999, 249-267 BOD2000-1371	N	-
Phae, C.-G., Sasaki, M., Shoda, M. and Kubota, H.	AIIB-7	1990	Characteristics of Bacillus subtilis Isolated from Composts Suppreeing Phytopthogenic Microorganisms. not GLP, published Soil Sci. Plant Nutr., 36, 4, 1990, 575-586 BOD2000-1368	N	-

[33] Only notifier listed

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[34]
Priest, F.G.	AIIB-7	1993	1. Systematics and Ecology of Bacillus. not GLP, published Journal of General Microbiology, 1993, 3-16 BOD2000-1370	N	-
Siala, A. and Gray, T.R.G.	AIIB-7	1974	Growth of Bacillus subtilis and Spore Germination in Soil Observed by a Fluores-cent-antibody Technique. not GLP, published Journal of General Microbiology, 81, 1974, 191-198 BOD2000-1369	N	-
van Elsas, J.D., Dijkstra, A.F., Govaert, J.M. and van Veen, J.A.	AIIB-7	1986	Survival of Pseudomonas fluorescens and Bacillus subtilis introduced into two soils of different texture in field microplots. not GLP, published FEMS Microbiology Ecology, 38, 1986, 151-160 BOD2000-1365	N	-
van Elsas, J.D., Govaert, J.M. and van Veen, J.A.	AIIB-7	1987	Transfer of Plasmid pFT30 Between Bacilli in Soil as Influenced by Bacterial Population Dynamics and Soil Conditions. not GLP, published Soil Biol. Biochem., 19, 5, 1987, 639-647 BOD2000-1367	N	-

Codes of owner

QST: AgraQuest, Inc.

[34] Only notifier listed

A.9 Ecotoxicology (Annex IIB 8; Annex IIIB 10)

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[35]
Adelberger, I.	AIIA-8.3.2	2000	QST 713 TP: Toxicity to the Predatory mite, Typhlodromus pyri Scheuten (Acari, Phytoseiidae) in the Laboratory. 99431/01-NLTp GLP, unpublished ANA2000-898	Y	QST
Asaka, O.; Ano, T.; Shoda, M.	AIIIA-10.6.1	1996	Persistence of Bacillus subtilis RB14 and its derivative strain in soil with respect to the lpa-14 Gene. not GLP, published J. of Fementation and Bioengineering, 81, 1, 1996, 1-6 ARW2000-129	N	-
Barrett et al.	AIIIA-10.6.1	1994	Guidance document on regulatory testing procedures for pesticides and non-target arthropods. Escord Workshop held at Wageningen 28-30 march 1994. not GLP, published 1994 ARW2000-130	N	-
Dengler, D.	AIIB-8.2	2000	Testing of toxic effects of QST 713 TP on the single cell green alga Scenedesmus subspicatus. 99431/01-AASs GLP, unpublished WAT2000-900	Y	QST
Drottar, K.R., Krueger, H.O.	AIIB-8.2	1998	Bacillus subtilis: A 21-day life-cycle toxicity and pathogenicity test with the cladoceran (Daphnia magna). 489A-102B GLP, unpublished WAT2000-899	Y	QST

[35] Only notifier listed

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[36]
Drottar, K.R., Krueger, H.O.	AIIB-8.2	1998	Bacillus subtilis: A 48-hour static acute toxicity test with the cladoceran (Daphnia magna). 489A-103 GLP, unpublished WAT2000-898	Y	QST
Drottar, K.R., Krueger, H.O.	AIIB-8.2	1998	Bacillus subtilis: A five-concentration toxicity and pathogenicity test with the rainbow trout (Oncorhynchus mykiss). 489A-101 GLP, unpublished WAT2000-897	Y	QST
Elsas v. J.D.; Dijkstra, A.F.; Govaert, J.M.; Veen v., J.A.	AIIIA-10.6.1	1986	Survival of pseudomonas fluorescens and bacillus subtilis introduced into two soils of different texture in field microplots. not GLP, published FEMS Microbiology Ecology, 38, 1986, 151-160 ARW2000-131	N	-
Elsas v., J.D.; Govaert, J.M. ; Veen v., J.A.	AIIIA-10.6.1	1987	Transfer of plasmid pFT30 between bacilli in soil as influenced by bacterial population dynamics and soil conditions. not GLP, published Soil Biol. Biochem., 19, 5, 1987, 639-647 ARW2000-132	N	-
EPA	AIIIA-10.6.1		Final decision document, TSCA section 5 (H) (4) exemption for Bacillus subtilis + Attachment 1. not GLP, published ARW2000-134	N	-
Foster, J.W.; Grimes, J. and Beavers, J.B.	AIIA-8.1	1998	Bacillus subtilis: An avian oral pathogenicity and toxicity study in the northern bobwhite. 489-101 GLP, unpublished AVS2000-98	Y	QST

[36] Only notifier listed

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[37]
Hoxter, K., Palmer, S., Krueger, H.	AIIA-8.3.2	1998	A dietary pathogenicity and toxicity study with the Parasitic Hymenoptera (Nasonia vitripennis). 489-105A GLP, unpublished ANA2000-896	Y	QST
Hoxter, K., Palmer, S., Krueger, H.	AIIA-8.3.2	1998	A dietary pathogenicity and toxicity study with the Green Lacewing Larvae (Chrysoperla carnea). 489-104 GLP, unpublished ANA2000-895	Y	QST
Hoxter, K., Palmer, S., Krueger, H.	AIIA-8.3.2	1998	A dietary pathogenicity and toxicity study with the Ladydird Beetle (Hippodamia convergens). 489-103B GLP, unpublished ANA2000-894	Y	QST
Hoxter, Kim-berly A.;Palmer, Susanne J.; Krueger, Henry O.	AIIIA-10.4	1998	Bacillus subtilis: A Dietary pathogenicity and toxicity study with the honey bee (Apis mellifera). 489-102C GLP, unpublished BIE2000-27	Y	QST
Phae, C.-G., Sasaki, M.; Shoda, M.; Kubota, H.	AIIIA-10.6.1	1990	Characteristics of Bacillus subtilis isolated from composts suppressing phytopatho-genic microorganisms. not GLP, published Soil Sci Plant Nutr., 36, 4, 1990, 575-586 ARW2000-136	N	-
Schuld, M.	AIIA-8.3.2	2000	QST 713 TP: Acute Toxicity to the Aphid Parasitoid, Aphidius rhopalosiphi (Hymenoptera, Braconidae) DeStefani-Perez in the Laboratory. 99431/01-NLAp GLP, unpublished ANA2000-897	Y	QST

[37] Only notifier listed

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[38]
Siala, A. Gray, T.R.G.	AIIIA-10.6.1	1974	Growth of bacillus subtilis and spore germination in soil observed by a fluorescent-antibody technique. not GLP, published J. of General Microbiology, 81, 1974, 191-198 ARW2000-137	N	-
Thobor, C.	AIIB-8.2	2000	Bacillus subtilis (150 g/kg technical powder) & Serenade TM WP (ai: 100 g/kg wettable powder) Summary of toxicity, pathogenicity and infectivity to non-target organisms, risk assessment and overall evaluations. AB981202-EU09/02 not GLP, unpublished WAT2000-896	N	QST

Codes of owner

QST: AgraQuest, Inc.

[38] Only notifier listed

Monograph

15 May 2001

Bacillus subtilis
strain QST 713

Volume 3

Annex B

Summary, Scientific
Evaluation and Assessment

Rapporteur Member State: Germany

Contents

Annex B

Bacillus subtilis strain QST 713

B-1: Identity

B.1 Identity

B.1.1 Identity of the micro-organism (Annex IIB 1)

B.1.1.1 Name and address of applicant(s) for inclusion of the organism in Annex I (Annex IIB 1.1)

Name:	AgraQuest, Inc.
Address:	1530 Drew Avenue Davis, CA 95616 U.S.A.
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Fax:	001-530-750 01 53
e-mail:	jrfox@agraquest.com
Contact Person:	Dr. Jennifer Ryder Fox
Position:	Director of Regulatory Affairs

Representative in Europe:

Name:	GAB Consulting GmbH
Address:	Hinter den Höfen 24 21 769 Lamstedt Germany
Contact Person:	Christina Thobor
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Fax:	+49-(0)4773- 8889-20
e-mail:	christina.thobor@GAB-consult.de

B.1.1.2 Manufacturer or manufacturers of the organism (Annex IIB 1.2)

Name:	AgraQuest, Inc.
Address:	1530 Drew Avenue Davis, CA 95616 U.S.A.
Phone:	001-530-750 01 50
Fax:	001-530-750 01 53
e-mail:	jrfox@agraquest.com
Contact Person:	Dr. Jennifer Ryder Fox
Position:	Director of Regulatory Affairs

Contract large scale producer:

Name:	FermPro Manufacturing LP
Address:	PO Box 5000 Kingstree, SC 29556 U.S.A.
Phone:	001-843-382-8485

B.1.1.3 Name and species description (Annex IIB 1.3)

B.1.1.3.1 Scientific name and taxonomic grouping (Annex IIB 1.3)

The species *Bacillus subtilis* strain QST 713 is a bacterium with an ellipsoidal endospore which does not extend the mother cell. The first description of the species is from Cohn (1872). Ehrenberg (1835) formerly described this species as *Vibrio subtilis* (Gordon et al., 1973). The genus *Bacillus* (rod-shaped, aerobic and facultative anaerobic, endospore-forming, ~ 60 species) (Priest, 1993) belongs to the family Bacillaceae (aerobic, saprophytic, endospore-forming) among the group gram-positive eubacteria (Schlegel, 1985).

B.1.1.3.2 Definition of the micro-organism

Characterisation of *B. subtilis*, strain QST 713 - which is identical with strain AQ 713:

- Gram-positive, motile rod measuring 1,0 µm x 3-5 µm with peritrichous flagella.

Colonial morphology varies with the different media employed for plating analysis. Besides the basically relevant positive Catalase reaction inherent to all *Bacillus* species, further biochemical key parameters identifying strain QST 713 of *B. subtilis* are e.g.: positive Voges-Proskauer reaction, growth in 7 % NaCl, and Casein decomposition (ATCC report, 1997, BMF2000-56).

Using the available morphological, physiological and biochemical data, the American Type Culture Collection clearly identified the strain AQ 713 as *Bacillus subtilis*. The strain AQ 713 is identical with strain QST 713.

This strain originates from a naturally occurring wild type, isolated from soil in California (USA) in 1995. Isolation of *Bacillus* species generally includes heat treatment of the suspended soil sample and subsequent streak plating using enrichment media (Slepecky, 1992)

The strain QST 713 has been added to the internationally accepted Agriculture Research Culture Collection (NRRL), Illinois, USA, code number NRRL B-21661.

B. subtilis is a genetically and physiologically well studied bacterium – second to *E. coli*- and regarded as non-pathogenic (e.g. Harwood, 1989, BMF 2000-59).

B.1.1.3.3 Appropriate test procedures and criteria used for identification

The isolate is identified by using classical morphological (cellular and colonial morphology), physiological and biochemical criteria. Biochemical key parameters characterise different *Bacillus* species and provide useful identification tools, as e.g. basically described by Slepecky (1992).

B.1.1.3.4 Common name or alternative and superseded names and code names used during the development (Annex IIB 1.3)

Bacillus subtilis, strain QST 713 is identical with strain AQ 713.

B.1.1.4 Composition of the material used for manufacturing of formulated products (Annex IIB 1.4)

B.1.1.4.1 Content of the micro-organism (Annex IIB 1.4.1)

The content of pure micro-organism in QST 713 Technical is 14.6 % by weight on average, and in terms of colony forming units 5 x 10^{10} cfu/g are stated (Cunningham Hilbig, 2000, BMF 2000-64). The technical product (powder) was proved to contain in minimum 9,9 x 10^{6} cfu/g of the active ingredient, *B. subtilis*, thus meeting the required minimum concentration for end use specification; inert ingredients consist of *B. subtilis* fermentation solids and/ or solubles and residual moisture (Bellet, 1998, BMF 2000-66).

Confidential information, see Annex C.

B.1.1.4.2 Identity and content of impurities, additives, extraneous micro-organisms (Annex IIB 1.4.2)

The end-product QST 713 Technical was determined to be 100 % microbiologically pure for *B. subtilis* (Gingras, 1998, BMF 2000-65, Lot Characterisation of *Bacillus subtilis* Strain QST 713).

Confidential information, see Annex C.

B.1.1.4.3 Analytical profile of batches (Annex IIB 1.4.3)

Confidential information, see Annex C.

B.1.2 Identity of the plant protection product (Annex IIIB 1)

B.1.2.1 Applicant (Annex IIIB 1.1)

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Davis, CA 95616
U.S.A
Contact Person: Dr. Jennifer Ryder Fox
Phone : 001-530-750 01 50
Fax : 001-530-750 01 53
e-mail: jrfox@agraquest.com

B.1.2.2 Manufacturer of the preparation and the micro-organism(s) (Annex IIIB 1.2)

Name: AgraQuest, Inc.
Address: 1530 Drew Avenue
Davis, CA 95616
U.S.A
Contact Person: Dr. Jennifer Ryder Fox
Phone : 001-530-750 01 50
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Kingstree, SC 29556
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Phone: 011-843-382-8485

B.1.2.3 Trade name or proposed trade name, and manufacturer's development code number (Annex IIIB 1.3)

Trade name: Serenade™ WP
Development code number: QRD 133 WP

B.1.2.4 Detailed quantitative and qualitative information on the composition of the preparation (Annex IIIB 1.4)

Confidential information, see Annex C

B.1.2.5 Physical state and nature of the preparation (Annex IIIB 1.5)

WP (wettable powder)

B.1.2.6 Function (Annex IIIB 1.6)

Fungicide, bactericide

B.1.3 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
AIIB-1.3; AIIB-2.5; AIIB-4.1	Anonymous	1997	Project Report SC 3817 - Bacterial Characterization ATCC. not GLP, unpublished BMF2000-56	Y	QST
AIIB-1.3; AIIB-2.1; AIIB-2.5	Gordon, R.E., Haynes, W.C. and Pang, C.H.-N.	1973	The Genus Bacillus. not GLP,published Agricultural Research Service United States Department of Agriculture Washington D.C. Agriculture Handbook, 427, 1973, 36-253 BMF2000-54	N	-
AIIB-1.3; AIIB-2.1; AIIB-2.4; AIIB-2.6; AIIIB-4.4	Harwood, C. R.	1989	Introduction to the Biotechnology of Bacillus. not GLP,published Bacillus, The University of Newcastle upon Tyne, UK Plenum Press, 1989, 1-4 BMF2000-59	N	-
AIIB-1.3; AIIB-2.1; AIIB-2.3; AIIB-2.4; AIIB-2.5; AIIB-2.6; AIIB-4.2	Priest, F. G.	1993	Systematics and Ecology of Bacillus. not GLP,published in: Bacillus subtilis and other gram-positive bacteria American Society of Microbiology, Washington D.C. (ed.), 1993, 3-16 BMF2000-57	N	-
AIIB-1.3; AIIB-2.1; AIIB-2.4	Schlegel, H.G.	1985	Allgemeine Mikrobiologie. not GLP,published Georg Thieme Verlag Stuttgart New York, 1985, 70-411 BMF2000-55	N	-
AIIB-1.3; AIIB-2.4; AIIB-4.1	Slepecky, R. A.	1992	What is a Bacillus?. not GLP,published in: Biology of Bacilli (Chapter 1) Doi, R. H. and Mc Gloughlin, M. (eds.) Application to Industry Buttenworth-Heinemann, Boston, 1992, 1-21 BMF2000-58	N	-

[1] Only notifier listed

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
AIIB-1.4; AIIB-2.1	Bellet, E. M.	1998	Manufacturing and Analytical Data for QST 713 Technical. EPA Reg. No.: 69592-X GLP, unpublished BMF2000-66	Y	QST
AIIB-1.4	Cunningham Hilbig, L.	2000	QST 713 Technical Powder - Confidential Statement of Formula. not GLP, unpublished BMF2000-64	Y	QST
AIIB-1.4	Gingras, B. A.	1998	Lot Characterisation of Bacillus subtilis strain QST 713. L08726 SN1 GLP, unpublished BMF2000-65	Y	QST
AIIIB-1.4	Cunningham Hilbig, L.	2000	SERENADE WP-CONFIDENTIAL STATE-MENT OF FORMULA. not GLP, unpublished PHY2000-715	Y	QST
AIIIB-1.4	Cunningham Hilbig, L.	1999	MANUFACTURING PROCESS FOR SE-RENADE WP FROM QST 713 STRAIN OF DRIED BACILLUS SUBTILIS. not GLP, unpublished PHY2000-714	Y	QST
AIIIB-1.4	Gingras, B. A.	1998	LOT CHARACTERIZATION OF QST 713STRAIN OF DRIED BACILLUS SUBTLS WITH RESIDUAL FERMENTA-TIO MEDIA IDENTIFIED AS QST 713 WP. GLP, unpublished PHY2000-716	Y	QST
AIIIB-1.4	Gordon, R. E.	1973	THE GENUS BACILLUS. not GLP,published , 427, 1973, pp.36-253 PHY2000-717	N	-

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
AIIIB-1.4	Priest, F.G.	1993	SYSTEMATICS AND ECOLOGY OF BACILLUS IN: BACILLUS SUBTILIS AND OTHER GRAM_POSITIVE BACTERIA. not GLP,published , 1993, pp.3-16 PHY2000-718	N	-
AIIIB-1.4	Schlegel, H.G.	1985	Die Zelle und Ihre Struktur; Das System der Prokapyoten; Unvollständige Oxydationen; Abbau von Naturstoffen. not GLP,published PHY2000-719	N	-

Codes of owner

QST: AgraQuest, Inc.

Annex B

Bacillus subtilis strain QST 713

B-2: Biological properties of the organism

B.2 Biological properties of the organism - Technical properties of the preparation

B.2.1 Biological properties of the micro-organism (Annex IIB 2)

B.2.1.1 History of the micro-organism and its uses. Natural occurrence and geographical distribution (Annex IIB 2.1)

B.2.1.1.1 Historical background (Annex IIB 2.1.1)

Bacillus subtilis was discovered in the late 19th century and originally served as the type species of the newly introduced genus *Bacillus*, followed by decades of confusion and inconsistency concerning the identification and nomenclature of species and strains (Gordon *et al.*, 1973). As a result numerous, mostly poorly described species were introduced (Priest, 1993).
In 1936 the International Congress for Microbiology adopted the so-called "Marburg strain" as the neotype of *B. subtilis* (Gordon *et al.*, 1973).
The taxonomic studies of N. R. Smith and co-workers in 1946, later continued by Gordon *et al.* (1973), strictly reduced the number of species comprising the genus *Bacillus* based upon comparative identification of more than 1000 strains. This work presents the framework of the currently valid systematic of the genus *Bacillus* (Priest, 1993), published in Bergey's Manual of Systematic Bacteriology (Sneath, 1986).
The central criterion for identification of *Bacillus* species is the endospore-morphology, additional criteria are physiological characteristics (Gordon *et al.*, 1973), DNA-DNA-hybridisation (Priest, 1993; Seki *et al.*, 1975) and characterisation by pyrolysis-gas chromatography (O'Donell *et al.*, 1980, BMF 2000-72).
To the present day the taxonomy of the heterogeneous genus *Bacillus* remains a point of discussion:
Priest (1993) suggests to divide the genus into five distinct groups (also for ease of identification of new isolates). Comparatively low DNA homology indexes between certain strains of *B. subtilis* together with other indications strongly suggested to divide the species into two different ones, corresponding to two distinguishable groups within the species (Seki, et al 1975).

Commercial uses and research:

B. subtilis is relevant as a model-organism for cell-biological research and has a potential as a commercial producer of the products of genetic engineering (Harwood, 1989; BMF 2000-59).
Traditional uses of *B. subtilis*, based upon empirical knowledge, concern fermented foods, i.e. the Japanese soybean product "Natto" and fermented cacao beans yielding "Cocoa" (Priest, 1993) – indicating the non-pathogenic character of these bacteria.
The capability of *B. subtilis* (and other bacteria of this genus) to produce exo-enzymes acting as proteases or cellulases is commercially used e.g. for tannery and for the production of additives for detergents (Schlegel, 1985) and in food industry (Priest, 1989, BMF 2000-69).
B. subtilis also produces surface active compounds of commercial value, like surfactin (Priest, 1989, BMF 2000-69).
Certain *B. subtilis* strains producing compounds with bactericidal action against some human pathogens stimulate the development of new drugs: Zimmermann *et al.* (1987) found two

relevant strains of *B. subtilis*, not suggesting to use the bacteria as potential agents but to employ the produced antibiotics as model-molecules.
Other scientists investigate the potential use of antifungal compounds as biological control agents against plant pathogens in agriculture (Citernesi *et al.*, 1994), the technical aspects of their exploitation (Ohno *et al.*, 1993; Ohno *et al.*, 1995, BMF 2000-73; Phae and Shoda, 1991; Pusey *et al.*, 1988) and genetic engineering to improve product yields (Matsuno, *et al.*, 1992).

In agricultural research the phenomenon of disease-suppressive soils led to the examination of the role of native microflora as one potential contributor (Alabouvette and Lemanceau, 1998). In the past decades the effects of various strains of *B. subtilis* on fungal growth have extensively been studied *in vivo* and *in vitro*, and fungicidal or at least suppressive activity against a variety of fungal diseases was determined, e.g. against:

Monilinia fructicola causing peach brown rot (Pusey and Wilson, 1984; Mc Keen *et al.*, 1986)
Penicillium expansum causing blue mold on apples (Sholberg *et al.*, 1995)
Fusarium wilt of tomato (Hiraoka *et al.*, 1992),
Rhizoctonia solani, *Streptomyces scabies*, *Erwinia carotovora* spp. (potato), *Sclerotinia sclerotiorum* (sunflower) (Schmiedeknecht *et al.*, 1998),
Fusarium oxysporum (cyclamen) (Phae *et al.*, 1990)
Ophiostoma ulmi causing the Dutch elm disease (Eshita *et al.*, 1995)

However, individual strains may differ in their antifungal spectrum.

As a side-effect of research on *B. subtilis* its beneficial effect on plant growth, early development of seedlings, and crop yield has led to new fields of application as growth stimulator (Kilian *et al.*, 1998, BMF 2000-86; Marten *et al.*, 1998; Kloepper *et al.*, 1989). Glick *et al.* (1999, BMF 2000-89) list *B. subtilis* as one of numerous soil inhabiting bacteria that can function as plant growth-promoter and state that continuously updated information, including references to the literature on plant growth-promoting bacteria, may be found on the Internet at www.ag.auburn.edu/pgpr/. *B. subtilis* strain FZB 24 is marketed as a plant strengthener in Germany.

B.2.1.1.2 Origin and natural occurrence (Annex IIB 2.1.2)

B. subtilis is an ubiquitous -not geographically restricted- inhabitant of the soil, from which it is spread to associated environments, including plants and plant materials (straw, composts*), foods (cereals, esp. dried spices), animals and their faeces (by ingestion of spores) and is also naturally found in aquatic environments (fresh water, estuarine and coastal waters) (Priest, 1993; Priest, 1989, BMF 2000-90; *Phae *et al.*, 1990). Although *B. subtilis* is commonly found in soil it occurs in almost any environment, including niches in kitchen and bathrooms (Boer and Diderichsen, 1991).

The magnitude of occurrence of *B. subtilis* in the soil is not definitely stated in the supplied literature. Priest (1989, BMF 2000-90) states that the reservoir of *Bacillus* species is the soil and generally, soils of low organic content have a restricted flora dominated by *B. subtilis*, *B. licheniformis* and *B. cereus*. However, with increasing fertility a wider range of species is encountered. Indications for the general presence of *Bacillus* species can be derived from high levels of presumably soil-born *Bacillus* spp. spores in straw approaching 10^5 cfu/g (Flannigan,

1987 cited in Priest, 1989, BMF 2000-90), and from the high numbers of *Bacillus* spp. found in coastal waters (where they constitute up to 20 % of total bacterial population) and from the major contribution of their endospores in estuarine and coastal sediments (reaching up to 80 % of the heterotrophic flora) (Priest, 1993).

Contrary to the above cited statement of Priest, Asaka *et al.* (1996) state that this bacterium is not regarded as a typical soil inhabitant. However, most references, including those of known *Bacillus* specialists state that the soil is the reservoir of *B. subtilis* and close relatives (Priest, 1993). In soil the inactive spore state is prevailing unless a soil has recently been amended with fresh organic matter (EPA, 1997).

Isolation of *Bacillus* species generally includes heat treatment of the suspended soil sample and subsequent streak plating using enrichment media (Slepecky, 1992). Agra Quest, Inc. isolated the QST 713 strain of *B subtilis* in 1995 from soil in California. The QST 713 strain was screened and fungicidal activity was confirmed (Bellet, 1998, BMF 2000-66).

B.2.1.2 Information on target organism(s) (Annex IIB 2.2)

B.2.1.2.1 Description of the target organism(s) (Annex IIB 2.2.1)

The envisaged target organisms are fungal and bacterial plant pathogens. The fungal pathogens are *Venturia inaequalis* (causing scab of apple), *Monilinia* spp. (causing fruit rots of stone fruits), *Uncinula necator* (causing powdery mildew of grapes), *Botrytis cinerea* (causing bunch rot of grapes) and *Bremia lactucae* (causing downy mildew on lettuce). The bacterial plant pathogen is *Erwinia amylovora* (causing fire blight of apples and pears).

B.2.1.2.2 Mode of action (Annex IIB 2.2.2)

Serenade^{TM} WP provides protection against several fungal plant pathogens and against bacterial vascular necrosis caused by *Erwinia amylovora*. The active ingredient, *B. subtilis*, is primarily a soil inhabitant but has been found in a variety of habitats world-wide. Strains of *B. subtilis* are known to be antagonistic towards a range of fungal plant pathogens. However, the mechanism for control of pathogens and the modes of action involved are not clearly understood for each host/ pathogen system.

In principle, the antagonism may be based on several mechanisms, including nutrient competition, site exclusion, attachment of the bacteria to the fungal pathogen, induction of physiological resistance responses in the host plant, and antibiosis caused by the action of bioactive compounds (Butt *et al.*, 1998).

Attachment of QST 713 strain *B. subtilis* to the fungal appressorium of grape powdery mildew is visualised in Scanning Electron Micrographs (SEM). The appressorium is disrupted and disintegrated in the presence of *B. subtilis*. This indicates a fungicidal action of QST 713.

The induction of chitinase and ß-glucanase – hydrolytic enzymes which decompose constituents of fungal cell walls – by QST 713 strain of *B. subtilis* further indicates direct antagonism of this strain against fungal plant pathogens (Jacobsen and Zidack, 2000).

In addition to antagonism / competition, results of inoculation experiments indicate that *B. subtilis* induces resistance in sugar beet plants: spraying of leaves with QST 713 prior to

inoculation with the pathogen *Erwinia caratovora* by injection into leaf veins resulted in reduced levels of disease and enhanced peroxidase activity as compared to the controls (Jacobsen and Zidack, 2000).

It can be concluded that Serenade^{TM} WP has both direct and indirect modes of action. Its active agent *B. subtilis* QST 713:

- inhibits attachment of the plant pathogen to the leaf by forming a physical barrier and/or produces a zone of inhibition restricting growth of disease causing pathogens,
- competes for space and nutrients with microbial plant pathogens,
- stops plant pathogen spores from germinating,
- disrupts the germ tubes,
- triggers plant defence mechanisms.

Review of literature on modes of action in pathogen control and relevant aspects for *B. subtilis*:

B. subtilis acts fungistatic/fungicidal against a broad spectrum of fungal plant pathogens, as mentioned under Point 2.1.1.1. Anti-fungal activity of *B. subtilis* or its metabolites has generally been evaluated by measuring growth inhibition *in vitro* (e.g. Eshita *et al.*, 1995; Klich *et al.*, 1991; Hiraoka *et al.*, 1992). Sometimes additional *in vivo* tests were performed (effects on infection by soil-born fungi: Loeffler *et al.*, 1986; roots growing in sterile soil: Citernesi *et al.*, 1994; stored apples: Sholberg *et al.*, 1995; peaches: Mc Keen *et al.*, 1986; leaf and pot tests: Phae *et al.*, 1990).

Fungicidal action of *B. subtilis* has been reported towards yeast cultures (Besson et al 1984).
According to Campell's review on biocontrol agents (1989, BMF 2000-100) the germination of *Botrytis* spores on the leaf surface is inhibited in the presence of various bacteria and yeasts by nutritional competition, and the author also explains the phenomenon of induced systemic resistance towards pathogens: an initial contact with a non-pathogenic species may elicit a resistance response of the plant via a systemically acting chemical signal. For strain QST 713 of *B. subtilis* the induction of biochemical resistance responses in sugar beet was indicated by increased peroxidase activity (Jacobsen and Zidack, 2000).

Marten *et al.*, (1998) explain the antagonistic effect of applied *B. subtilis* preparations against phytopathogens (*Rhizoctonia solani* and *Fusarium oxysporum*) with several involved mechanisms, including protease-activity (causing lysis) and competition due to production of siderophores.

Competition for nutrients (esp. carbon) occurs among the saprophytic members of the micro-flora within the natural habitat, the soil, and in the rhizosphere. Successfully competing bacteria inhibit fungal spore germination (fungistasis) and therefore competition is believed to present one potential mode of action in the suppression of fungal plant diseases like *Fusarium* wilts (Alabouvette and Lemanceau, 1998). Together with other factors competition promotes vital growth and development of the roots (Kilian *et al.*, 1998, BMF 2000-86).

For strain QST 713 of *B. subtilis* the mode of action against fungal phytopathogens was determined to be principally based upon colonisation of the leaf surface and the fungal

pathogen and additionally based on the induction of biochemical resistance responses in the plant (Jacobsen and Zidack, 2000).

B.2.1.3 Host specificity range and effects on species other than the target harmful organism (Annex IIB 2.3)

B. subtilis is not characterised by a distinct host specificity – in contrary e.g. to the biocontrol agent *Coniothyrium minitans* – since growth of *B. subtilis* is not dependent upon a host but upon supply with decomposable organic matter. The endospore is the prevalent stage of *B. subtilis* in all environmental compartments. *B. subtilis* is not geographically restricted (Priest, 1993).

Effects on non-target organisms:

Micro-organisms:
The potential suppression of mycorrhizal species was tested by applying the antimycotic molecule Iturin A 2, an antibiotic produced by certain strains of *B. subtilis*. In vitro the saprophytic growth of the fungus *Glomus mosseae* was inhibited by Iturin A 2, while in vivo, in the presence of the tomato host plant, the antibiotic did not restrict or impede the mycorrhizal symbiosis at any stage. Under field conditions Iturin A 2 inhibited the pathogen *Fusarium oxysporum* and effectively reduced the infection (Citernesi *et al.*, 1994).

Plants:
B. subtilis has been reported causing soft-rot of a halophytic species grown in Mexico and southern U.S.A. as an oilseed and forage crop, *Salicornia* (Stanghellini and Rasmussen, 1989) and in garlic cloves (Kararah *et al.*, 1985). However, there is no evidence for a general property or tendency of the species to attack plants or plant parts. Lack of pathogenicity is also proven by the use of *B. subtilis* preparations on different crops to promote growth and to improve yields (Kilian *et al.*, 1998, BMF 2000-86; Marten *et al.*, 1998; Schmiedeknecht *et al.*, 1998).

As stated by the applicant, in more than 400 field applications on trees and crops no negative impacts by strain QST 713 of *B. subtilis* were observed.

Animals:
Certain strains of *B. subtilis* isolated from diseased mosquito larvae were infectious and pathogenic towards exposed mosquito larvae – lowest LC_{50} was 1×10^3 cfu/ml – suggesting a potential use as insecticide to control malaria (Gupta and Vyas, 1989). The same report states mammalian toxicity of two out of four tested strains towards mice after intraperitoneal injection. The authors concluded that the non-pathogenic strains were safe for field application.

No pathogenic or toxic impacts of strain QST 713 of *B. subtilis* on mammals can be concluded from the toxicological studies described in chapter B.6.5. In addition, no hazardous effects of strain QST 713 of *B. subtilis* were observed in testing non-target organisms (invertebrates, arthropods, fish and birds) that might be exposed to it under conditions of use (see chapter B.9).
In conclusion, adverse impacts and risks of field application of strain QST 713 of *B. subtilis* for exposed animals are most unlikely.

B.2.1.4 Development stages / life cycle of the micro-organism (Annex IIB 2.4)

As a saprophytic micro-organism of the soil *B. subtilis* contributes to the mineralisation of organic molecules due to break down by secreted proteases and amylases (Schlegel, 1985). Some of these enzymes are responsible for the occurrence of soft-rot disease caused by *B. subtilis* in several crops (Stanghellini and Rasmussen, 1989; Kararah *et al.*, 1985) and are commercially exploited (Harwood, 1989, BMF 2000-59).

Life-cycle of *Bacillus*:
All spore-formers, including members of the genus *Bacillus*, undergo a cycle consisting of several discernible phases: germination, outgrowth, multiplication, and sporulation (Figure B.2.1-1). The germination is the conversion of the quiescent and dormant spore to a metabolising cell capable of outgrowth. The primary cell formed at the end of outgrowth can, under some conditions, such as insufficient supply with nutrients, divide asymmetrically and proceed directly to sporulation (referred to as microcycle sporulation in Figure B.2.1-1). Under favourable conditions, such as availability of sufficient nutrients, the primary cell can divide symmetrically and proceed through many divisions before sporulating (Slepecky, 1992). A specific review on spore germination is given by Moir (1992).
The endospore plays a dominant role in the biology and the life-cycle of *B. subtilis* and its relatives (Priest, 1993). It is a dormant structure which enables the micro-organism to survive when environmental conditions turn unfavourable for vegetative growth and a vehicle for dispersal by dust and air streams, as it is easily blown up (Priest, 1993). The global distribution of *Bacillus* spp. may largely be derived from the endospore-forming capability. Basically the endospore is the most heat tolerant bacterial life-form, enduring temperatures >80 °C or even >100 °C (Schlegel 1985). The endospore does not present an obligate stage in the life-cycle, vegetative growth by cell-division may be predominant - or even the norm, unless e.g. lack of nutrients occurs (Priest, 1993). Studies on the population dynamics of *B. subtilis* applied to soil strongly suggest that generally *B. subtilis* occurs in the endospore form in soil (van Elsas *et al.*, 1986; Asaka *et al.*, 1996).
In a dry state endospores can remain viable for several years: after 50 years of storage of dry soil, 10 % of the spores retain their capability to germinate (Schlegel, 1985).

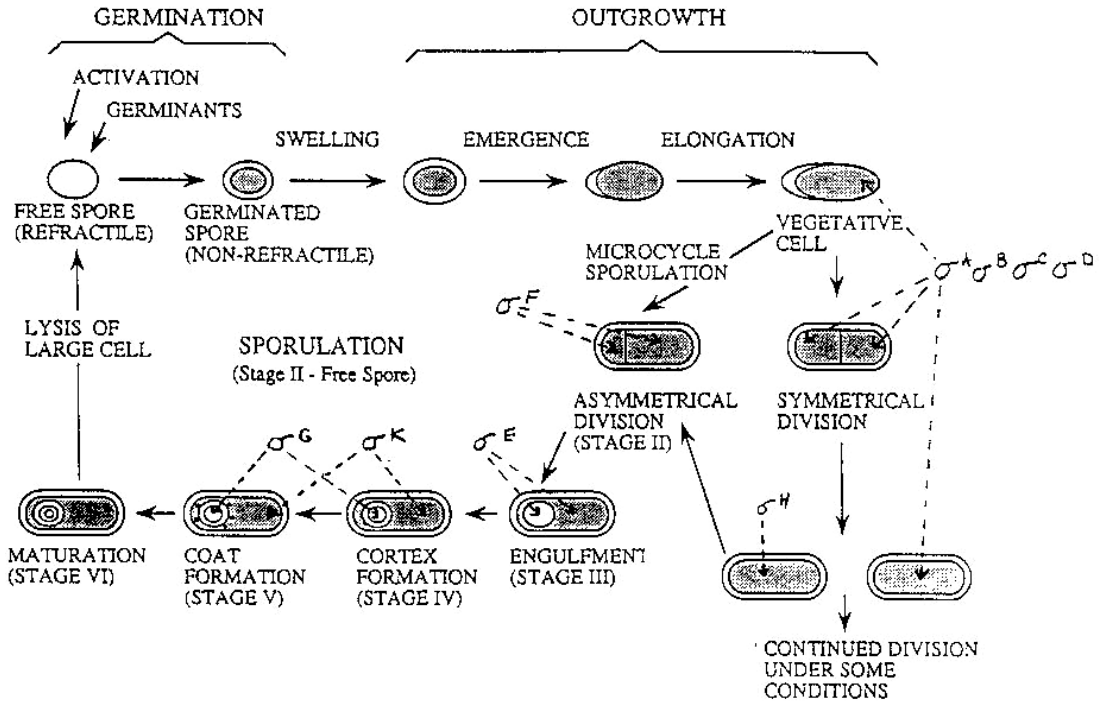

Figure B.2.1-1: Life-cycle of a typical spore-forming bacterium, where the various sigma factors* are involved as indicated (SLEPECKY 1992).

Sigma factors are sequence specific DNA binding proteins involved in DNA transcription

B.2.1.5 Infectivity, dispersal and colonisation ability (Annex IIB 2.5)

Several facts support an overall low significance of these aspects for *B. subtilis*:

- The global distribution of *B. subtilis* in any media (air/ soil/ water), organisms and on foodstuff
- The ease of dispersal of the endospore
- The status "generally regarded as safe" for *B. subtilis*, granted by the U.S. Food and Drug Administration (Harwood, 1989, BMF 2000-59; Priest, 1989 BMF 2000-69).
- The classification "Class 1 Containment" according to the European Federal Law of Biotechnology and "Class 1 Containment Agent" by the U.S. National Institute of Health (EPA, 1997)

Infectivity

Animals/ Humans:

Data about *B. subtilis* acting as a pathogen are rare. Gupta and Vyas (1989) reported insect- and mammalian-pathogenicity of some of the *B. subtilis* strains isolated from diseased mosquito larvae, causing mortality due to invasive infection (with subsequent decomposition of larvae) after ingestion by larvae or after intraperitoneal injection in mice, respectively. Evidence for *B. subtilis* -as one of several possible causative agents- causing bovine mastitis has been found in some cases (Fossum *et al.*, 1986).

The U.S. EPA considers risks from use of *B. subtilis* as low, but states immuno-compromised individuals inoculated with high numbers of the micro-organism may be susceptible to an infection (EPA 1997; Boer and Diderichsen, 1991).
According to de Boer and Diedrichsen (1991), *B. subtilis* is even consumed in large quantities in the Japanese food "Natto". It is therefore considered an opportunistic micro-organism with no pathogenic potential to humans. However, *B. subtilis* is virtually ubiquitous and it is therefore inevitable that it sometimes may be found in association with other micro-organisms in infected humans, but only patients treated with immunosuppressive drugs appear to be susceptible to infection with this otherwise harmless micro-organism. *B. subtilis* has been associated with some cases of food poisoning, which in part may be due to misclassification of *B. cereus*. Thus there are very few examples of *B. subtilis* strains as the confirmed causes of food poisoning. Finally, the U.S. EPA states that the only health problem in fermentation facilities may be sensitisation of workers to the *B. subtilis* derivative subtilisin, since this proteinaceous compound is capable of causing allergic reactions in individuals who are repeatedly exposed to it (EPA, 1997).
QST 713 strain of *B. subtilis* did not exert pathogenic or toxic impacts on mammals, as proved in relevant toxicological studies described in chapter B.6.11.
In addition, no hazardous effects of strain QST 713 of *B. subtilis* were observed in testing non-target organisms (invertebrates, arthropods, fish and birds) that might be exposed to it under conditions of use (see Chapter B.9).
In conclusion, adverse impacts and risks of field application of strain QST 713 of *B. subtilis* for exposed animals are not expected.

Plants:
Although a few studies relate incidence of soft-rot disease on certain crops or crop products to *B. subtilis*, there is no evidence for a general property or tendency of the species to attack plants or plant parts (see chapter B 2.1.3).

Dispersal routes

Endospores of *B. subtilis* may easily be distributed with soil or dust particles and via aerosols. Under conditions of use drift and spacious transport may occur with surface water and with the wind swirling up these particles or aerosols (Priest, 1993). During aerosolisation vegetative cells of *B. subtilis* are exposed to severe environmental stress factors (desiccation, UV-radiation, temperature), therefore survival of vegetative cells is limited. However, the ability of *B. subtilis* to survive in a broad habitat range and produce endospores suggest that this organism will survive after release (EPA, 1997).

Environmental requirements

Generally *B. subtilis* reproduces under aerobic conditions, although in the presence of glucose and nitrate anaerobic growth occurs (Priest 1993). *B. subtilis*, together with closely related species, is prevalent in soils, particularly in low-nutrient soils (Priest, 1993) and is dominating the restricted micro-flora of soils with low organic content (Priest, 1989, BMF 2000-90).
The endospore is the most important aspect of *Bacillus* ecology for several reasons. First, because heat treatment is the most common selective isolation procedure for the recovery of *Bacillus* spp. from the environment, most studies concentrate on the endospore to the exclusion of vegetative cells. Second, because the spore is a dormant structure of great longevity, ecological studies are often simply estimates of the accumulation of spores in an environment rather than an assessment of the contribution of the bacterium to the

environment. Nevertheless, a large number of spores of a particular species in a habitat is strongly indicative of previous or continuing growth and metabolism in that niche (Priest 1993). The reported predominance of *B. subtilis* and related Bacilli in soils may indicate low demands for nutrition of these bacteria or more probably the successful survival strategy rendered by sporulation. *B. subtilis* is reported to occur predominantly in the resting stage (endospore), unless fresh organic matter has been supplied to the soil (EPA, 1997). In any case, application of organic matter, e.g. manure, will support growth of existing *B. subtilis* populations.

A minimal medium for vegetative growth of *B. subtilis* has no vitamins and contains glucose and an ammonium salt as the sole sources of carbon and nitrogen (Sneath 1986). The influence of pH on growth of *B. subtilis* was tested by Sneath (1986). The pH-range for growth was found to be pH 5,5 to 8,5. The ATCC report (1997, BMF 2000-56) states growth of *B. subtilis* strain QST 713 at pH 4.5 and pH 6. In the Voges-Proskauer test the strain QST 713 growth at pH 6.6 – 7.5. Gordon *et al.* (1973) defined the temperature range for growth of various *B. subtilis* strains: maximum growth was achieved at 45 – 55 °C (one strain at 35 °C); the minimum temperature allowing growth was 5 to 20 °C. Metabolic activity of *B. subtilis* at low temperatures can be deduced from the report of Kilian et al. (1998, BMF 2000-153) who observed satisfactory activity of a yield-increasing strain on potatoes planted early in the spring. In liquid cultivation of *B. subtilis* optimal growth occurred at 37 to 42 °C and production of an antibiotic was also shown to be temperature dependent (Ohno *et al.*, 1993). The ATCC report (1997, BMF 2000-56) confirmed 55 °C as the maximal temperature permitting growth of strain QST 713 of *B. subtilis*, the lowest temperature tested allowing growth was 15 °C.

Application of endospore suspensions of *B. subtilis* to seeds alone or additionally to the growth substrate stimulated growth of pea seedlings under different temperatures (10 to 30 °C). With rising temperatures the population density increased both in the substrate and especially in the rhizosphere, whereas the degree of growth promotion remained unchanged. Thus, a correlation between growth promoting activity of *B. subtilis* and its population density could be established (Zimmer *et al.*, 1989).
B. subtilis added to a thermophilic composting reactor for sewage sludge survived and kept its antifungal activity against phytopathogenic fungi (Phae *et al.*, 1990).

In conclusion, several references indicate that *B. subtilis* will survive under a broad spectrum of environmental conditions. No negative impacts on Serenade efficacy in controlling crop diseases are anticipated under normal European growing season conditions (~ 10 to 30°C).

Colonisation ability

The efficacy of strain QST 713 of *B. subtilis* against phytopathogens is largely based upon colonisation of the leaf surface.
Tests on the survival of strain QST 713 of *B. subtilis* on pepper leaves were conducted under greenhouse conditions and demonstrated that cell numbers increased to day 5 followed by a sharp decline in the number of colony forming units (cfu). The results indicated that the employed strain was potentially able to survive for a couple of weeks under greenhouse conditions. The authors emphasise that survival may be different under summer field conditions, especially with regard to negative effects of ultra violet radiation (Yuan and Heins, 2000, BMF 2000-148).

There is little data support in the open literature concerning colonisation of foliage or above-ground parts by *Bacillus subtilis*, presumably due to its prime occurrence in soils. Sholberg *et al.* (1995) isolated several strains of *B. subtilis* from the surface of apples for screening of post-harvest disease control.

B.2.1.6 Relationships to known plant or animal or human pathogens (Annex IIB 2.6)

B. subtilis and close relatives are regarded as non-pathogenic micro-organisms and accepted as "organism GRAS" (generally regarded as safe) by the U.S. Food and Drug Administration. Other species of the genus *Bacillus* are known as toxin forming pathogens of vertebrates and arthropods (Harwood, 1989 BMF 2000-59):

B. anthracis	causes anthrax in humans and animals (Priest, 1993)
B. cereus	causes gastroenteritis (via food) and opportunistic infections (Priest, 1993)
B. thuringiensis	acts as an insect pathogen

Purity checks of the Technical product of *B. subtilis* strain QST 713 are continuously performed to exclude the presence of human pathogens and other contaminant micro-organisms (Bellet, 1998, BMF 2000-66; Gingras, 1998 BMF 2000-65).

In conclusion more information is necessary for the morphological differentiation of *B. subtilis* and the above indicated pathogenic *Bacillus* species.

B.2.1.7 Genetic stability and factors affecting it (Annex IIB 2.7)

Genetic stability
Studies in this respect have focussed on genetic engineering and instability problems of inserted DNA (Mountain, 1989). Research on basic genetic mechanisms has extensively made use of *B. subtilis* as a model system, e.g. to study plasmid stability characteristics. A number of studies investigated the fate of transferred recombinant or naturally occurring plasmids, and stability was shown to be influenced by environmental factors, the stage of the host cell and the plasmid size (Tokuda *et al.*, 1993; Leonhardt and Alonso, 1991; Ohno *et al.*, 1995, BMF 2000-164; Asaka *et al.*, 1993). To our knowledge studies on the genetic stability of natural traits related to the biocontrol activity of *B. subtilis* have not been performed.

Gene transfer
Unlike *Escherichia coli* cells *B. subtilis* does not exchange genetic material via true conjugation (Ferrari and Hoch, 1989). However van Elsas *et al.*, (1987) found evidence for conjugation between cells of *B. cereus* and *B. subtilis*. A natural way of DNA transfer is through bacteriophages, that may package some percent of the chromosomal DNA. Bacteriophages have been employed for analysis of the genetic structure of *B. subtilis* (Ferrai and Hoch, 1989).

Under natural conditions genetic exchange of chromosomal DNA (here: linked markers for antibiotic resistance) was shown to occur between strains of *B. subtilis* growing together in soil, presumably by transformation (Graham and Istock, 1979): within one week the mixed soil culture developed towards dominance of one phenotype based on one specific combination of transferred genes out of more than 100 initially present phenotypes.
Interspecies gene transfer has been proved by van Elsas *et al.* (1987), who suggested conjugation between *B. cereus* and *B. subtilis* as the transfer mechanism of a plasmid carrying

tetracycline resistance. The plasmid transfer from *B. cereus* to *B. subtilis* was detectable in sterile, nutrient amended soil at a frequency of 1 x 10^{-6}, but it turned negligible in non-sterile soil, unless bentonite clay was added which improved survival of *B. subtilis*. The plasmid transfer also declined with increasing temperature and moisture content.
Mainly, interspecies gene transfer is studied and achieved under specific laboratory conditions employed in genetic engineering, e.g. by manipulating membrane permeability and by using certain plasmid vectors – in this context Klier *et al.* (1983) reported successful transformation of genes between *B. subtilis* and *B. thuringiensis*.

Taken together, available knowledge indicates that gene transfer within *B. subtilis* or between *B. subtilis* and related species under natural conditions may be a rare event but can not be fully ruled out. In case of QST 713 this is not a problem because fermentation is started with pure cultures of QST 713. A transfer to QST 713 of genes governing undesirable properties can therefore be ruled out. Regarding a possible gene transfer after application it must be considered that QST 713 does not have undesirable trails, e.g. pathogenicity to humans, animals or plants.

B.2.1.8 Information on the production of metabolites (especially toxins) (Annex IIB 2.8)

B. subtilis produces different exo-enzymes contributing to the decay of organic matter. *B. subtilis* is known for producing lipopeptides such as iturins, plipastatins and surfactins (Manker, 2000). One extracellular enzyme is subtilisin. Although subtilisin has very low toxigenic properties, this proteinaceous compound is capable of causing allergic or hypersensitive reactions in individuals repeatedly exposed to it (EPA, 1997). However according to EPA (1997) *B. subtilis* does not produce significant quantities of extracellular enzymes or toxins and *B. subtilis* appears to have low degree of virulence to humans. The results of the submitted toxicological studies on rodents do not show evidence of toxin production of QST 713 strain of *B. subtilis* at relevant levels (see chapter B.6.5).

B.2.1.9 Antibiotics and other anti-microbial agents (Annex IIB 2.9)

Different antibiotic molecules were identified as products from different strains of *B. subtilis*. Antibiotic production is specifically related to the growth stage or age of a culture (Barr, 1975). It is often associated with the end of the logarithmic growth phase and the early stages of sporulation without being directly involved in sporulation, as proved by antibiotic deficient mutants that sporulated normally (Priest, 1989, BMF 2000-69; Feignier *et al.*, 1995).
Katz *et al.* (1977) listed and described several antibiotics produced by certain strains of *B. subtilis*, e.g. bacillomycin, bacillin, eumycin, fungistatin, iturin and mycobacillin. Numerous studies concentrated on one group of antibiotics, the iturins. Iturins consist of a cyclic octapeptide with a lipophilic β-amino acid side-chain of variable length, reported to directly interact with the cytoplasmic membrane and to be important for the displayed antifungal activities (Besson *et al.*, 1976; Besson *et al.*, 1979; Bland *et al.*, 1995; Citernesi *et al.*, 1994; Delcambe *et al.*, 1977; Klich *et al.*, 1991; Latoud *et al.*, 1987; Matsuno *et al.*, 1992; Thimon *et al.*, 1992).

Other antibiotics were defined as macrocyclic polyene lactone phosphate esters, isolated from fermentation broth of certain strains of *B. subtilis*. These antibiotics were shown to act antibacterial and to act against human pathogens (Zimmermann *et al.*, 1987). However, no antibiotics used in human or animal medicine are known to be produced by *B. subtilis*.

In the natural habitat spontaneous mutants of *B. subtilis* are reported to occur, exhibiting resistance towards antibiotics, e.g. towards streptomycin in strains isolated from composts (Phae *et al.*, 1990; Tokuda *et al.*, 1993).
The detailed genetic map of the *B. subtilis* chromosome, published in the book "Bacillus", edited by Harwood (1989, BMF2000-179), includes some genes participating in antibiotic resistance. Matsuno *et al.* (1992) located resistance genes on different plasmids isolated from compost bacteria by transferring each plasmid to competent and specifically prepared bacteria cells of *B. subtilis*. The plasmids carried resistance towards chloramphenicol, erythromycin, and tetracycline.

Bacillus species, including *B. subtilis*, were isolated from infected patients (mainly from their wound drainage) and found to be uniformly resistant to a variety of antibiotics (penicillin, ampicillin, oxacillin, methicillin and colistin (Ihde and Armstrong, 1973). *B. subtilis* strains isolated from milk of cows suffering from mastitis were resistant to streptomycin and tetracycline (Fossum *et al.*, 1986).

References about the clinical dimension and significance of these reported resistances are lacking. The cases of *B. subtilis* acting as a causal agent for disease or even causing mortality are rare and always associated with individuals showing immuno-suppression, while less severe infections by contamination of wounds are more likely to occur (Ihde and Armstrong, 1973; EPA, 1997). The commercial application of *B. subtilis* in the Japanese food "Natto" and the prevalence of its endospores (e.g. in spices) should be considered in evaluating the safety of this micro-organism.
The employment of *B. subtilis* as host for vaccine or diagnostic antigen production in medical applications supports the view that this bacterium has low risk potential for humans (Saris *et al.*, 1990, BMF2000-165 and BMF2000-166; Taira *et al.*, 1989).

The applicant stated that from complete chemical analysis of the strain QST 713 it can be concluded that no compounds are produced that are not well known in the literature. Based on a literature search the applicant stated further that the strain QST 713 does not produce metabolites that are used in human medicine (Manker, 2000).
The applicant stated further that no resistance genes against antibiotics which are used in animal or human medicine are inherent to strain QST 713 of *B. subtilis*. Spontaneous mutations within the seed vials used for fermentation are excluded since the culture is consistently stored at appropriate storing conditions (Bellet, 1998, BMF 2000-66).

In conclusion more detailed information is necessary regarding resistance of strain QST713 against antibiotics and about the antibiotics produced by it. *B. subtilis* strain QST 713.

B.2.2 Physical, chemical and technical properties of the plant protection product (Annex IIIB 2)

Product name: Serenade™ WP (containing 5 x 10^9 cfu/g product, WP)

Table B.2.2: Summary of the physical, chemical and technical properties of the plant protection product

Section (Annex point)	Study	Method	Results	Comment/Conclusion	Reference
B.2.2.1.1 (IIIB 2.1)	Appearance: colour	Visual assessment	Light to medium brown		Walter (2000) PHY2000-722
B.2.2.1.2 (IIIB 2.1)	Appearance: Odour		Earthlike		Walter (2000) PHY2000-722
B.2.2.2.1 (IIIB 2.2)	Effects of light, temperature and humidity		Physically stable at 40 °C for 8 weeks. The test substance was determined to be stable for one year when stored at warehouse (ambient) conditions. The 2 year study is currently under way.	Acceptable	Walter (2000) PHY2000-722 Gingras (1999) PHY2000-721
B.2.2.2.2 (IIIB 2.2)	Other factors affecting stability		No data submitted.		
B.2.2.3.1 (IIIB 2.3)	Explosive properties Lower dust explosive limit	EEC A 14 ASTM E1515	Non-explodable. 440 g/m³	Acceptable.	Anonymous (1999) PHY2000-720 Anonymous (2000) PHY2001-128

Section (Annex point)	Study	Method	Results	Comment/Conclusion	Reference
B.2.2.3.2 (IIIB 2.3)	Oxidising properties		No oxidising properties.	Acceptable.	
B.2.2.4.1 (IIIB 2.4)	Flash point		Not applicable.		
B.2.2.4.2 (IIIB 2.4)	Flammability	EEC A 10	No data submitted.	Study in progress.	Anonymous (1999) PHY2000-720
B.2.2.4.3 (IIIB 2.4)	Auto-flammability		No data submitted.	Study in progress.	
B.2.2.5.1 (IIIB 2.5)	Acidity/alkalinity		Not necessary.	Acceptable	
B.2.2.5.2 (IIIB 2.4)	pH of a 1 % aqueous solution	CIPAC MT 75	5.45 at 1 % concentration in distilled water; After accelerated storage for 8 weeks at 40 °C: 5.48 – 5.49 at 1 % concentration in distilled water	Acceptable	Walter (2000) PHY2000-722
B.2.2.6.1 (IIIB 2.6)	Kinematic viscosity		Not applicable.		
B.2.2.6.2 (IIIB 2.6)	Dynamic viscosity		Not applicable.		
B.2.2.6.3 (IIIB 2.6)	Surface tension	EEC A 5 ring tensiometer	37.7 mN/m at 20.1 °C and 0.1 % concentration	Acceptable	Walter (2000) PHY2000-723

Section (Annex point)	Study	Method	Results	Comment/Conclusion	Reference
B.2.2.7.1 (IIIB 2.7.1)	Wettability	CIPAC MT 53.3	Without swirling : < 17 min 20 s; With swirling: < 1 min 45 s After accelerated storage for 8 weeks at 40 °C: Without swirling: < 13 min 20 s With swirling: < 2 min 40 s	Preparing the broth may be difficult.Thorough stirring is needed.	Walter (2000) PHY2000-722
B.2.2.7.2 (IIIB 2.7.2)	Persistant foaming	CIPAC MT 47.2	Foam after 10 s: < 9 ml Foam after 1 min: < 5 ml Foam after 3 min: < 2 ml Foam after 12 min: 0 ml After accelerated storage for 8 weeks at 40 °C: Foam after 10 s: < 14 ml Foam after 1 min: < 10 ml Foam after 3 min: < 7 ml Foam after 12 min: 0 ml	Acceptable	Walter (2000) PHY2000-722
B.2.2.7.3.1 (IIIB 2.7.3)	Suspensibility	CIPAC MT 161	> 70 % before and after accelerated storage for 8 weeks at 40 °C:	Acceptable.	Anonymous (1999) PHY2001-65 Cunningham Hilbig, Ryder Fox (2000) PHY2001-133
B.2.2.7.3.2 (IIIB 2.7.3)	Spontaneity of dispersion		Not applicable.		

Section (Annex point)	Study	Method	Results	Comment/Conclusion	Reference
B.2.2.7.4 (IIIB 2.7.4)	Wet sieve test	CIPAC MT 59.3	0,078 % < 75 µm After accelerated storage for 8 weeks at 40 °C: 0,167 % < 75 µm	Acceptable.	Walter (2000) PHY2000-722
B.2.2.7.5.1 (IIIB 2.7.5)	Particle size distribution	OECD 110 DIN 66115	x_{90} = 14 µm x_{10} = < 1 µm	Acceptable.	Walter (2000) PHY2000-722
B.2.2.7.5.2 (IIIB 2.7.5)	Dust content		Not applicable.		
B.2.2.7.5.3 (IIIB 2.7.5)	Friability and attrition		Not applicable.		
B.2.2.7.6.1 (IIIB 2.7.6)	Emulsifiability, emulsion stability and re-emulsifiability		Not applicable.		
B.2.2.7.6.2 (IIIB 2.7.6)	Stability of dilute emulsion		Not applicable.		
B.2.2.7.7.1 (IIIB 2.7.6)	Flowability		Not applicable.		
B.2.2.7.7.2 (IIIB 2.7.7)	Pourability (rinsability)		Not applicable.		

Section (Annex point)	Study	Method	Results	Comment/Conclusion	Reference
B.2.2.7.7.3 (IIIB 2.7.7)	Dustability		Not applicable.		
B.2.2.8.1 (IIIB 2.8)	Physical compatibility with other products		With various fungicides, insecticides and adjuvants compatible.		Campbell, Cunningham-Hilbig (2000) PHY2000-726
B.2.2.8.2 (IIIB 2.8)	Chemical compatibility with other products				
B.2.2.8.3 (IIIB 2.8)	Biological compatibility with other products				
B.2.2.9 (IIIB 2.9)	Adherence and distribution to seeds		No seed dressing formulation.		

B.2.2.10 (IIIB 2.11): Summary and evaluation of data presented under points B.2.2.1 to B.2.2.9

Serenade™, a preparation with the active substance QST 713 strain of Bacillus subtilis is not explodable or flammable. It delivers an earthlike odour and is light to medium brown. The wetting of the water dispersible powder occurs slowly and requires thorough stirring of the dispersion. All other technical properties indicate that no problems will occur in practice, if the product is applied as recommended.
At room temperature this biological plant protection product is stable for at least one year. The two years study is in progress. Storage at 40 °C for 8 weeks has been determined not to alter physico-chemical and technical properties of this product.

B.2.3 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[2]
AIIB-1.4; AIIB-2.1	Bellet, E. M.	1998	Manufacturing and Analytical Data for QST 713 Technical. EPA Reg. No.: 69592-X GLP, unpublished BMF2000-66	Y	QST
AIIB-2.1	Kloepper, J.W., Lifshitz, R., Zablotowicz, R.M.	1989	Free-Living bacterial inocula for enhancing crop productivity. not GLP, published Tibtech, Vol. 7, 1989, 39-44 BMF2000-88	N	-
AIIB-2.1	O`Donnel, A.G., Norris J.R., Berkeley R.C.W., Claus D., Kaneko T., Logan N.A. and Nozaki R.	1980	Characterization of bacillus subtilis, Bacillus pumilus, Bacillus licheniformis, and Bacillus amyloliquefaciens by Pyrolysis Gas-Liquid Chromatography, Deoxyribonucleic Acid-Deoxyribonucleic Acid Hybridization, bio-chemical Tests, and API Systems. not GLP, published International J. of systematic Bacteriology Vol.30, No. 2, 1980, 448-459 BMF2000-139	N	-

[2] Only notifier listed

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[2]
AIIB-2.1	Ohno, A., Ano, T. and Shoda, M.	1995	Effect of temperature on production of lipo-peptide antibiotics, iturin A and surfactin by a dual producer, Bacillus subtilis RB14, in solid-state fermentation. not GLP, published J. of Fermentation and Bioengineering, Vol. 80, No. 5, 1995, 517-519 BMF2000-73	N	-
AIIB-2.1	Phae, C.-G. and Shoda, M.	1991	Investigation of optimal conditions for foam separation of iturin, an antifungal peptide pro-duced by Bacillus subtilis. not GLP, published J. of fermentation and Bioengineering, Vol. 71, No. 2, 1991, 118-121 BMF2000-75	N	-
AIIB-2.1	Pusey, P.L. and Wilson, C.L.	1984	Postharvest biological control of stone fruit brown rot by Bacillus subtilis. not GLP, published Plant Disease, Vol. 68, No. 9, 1984, 753-756 BMF2000-79	N	-
AIIB-2.1	Pusey, P.L. et al.	1988	Pilot tests for commercial production and ap-plication of Bacillus subtilis (B-3) for posthar-vest control of peach brown rot. not GLP, published Plant Disease, Vol. 72, No. 7, 1988, 622-626 BMF2000-76	N	-
AIIA-2.4; AIIB-2.1; AIIB-2.2	Alabouvette, C. and Lemanceau, P.	1998	Joint action of microbials for disease control (Article 8). not GLP, published Methods in Biotechnology, Vol. 5 Biopesticides: Use and Delivery, 1998, 117-135 BMF2000-78	N	-
AIIB-2.2	Besson, F., Peypoux, F., Quentin, M.J. and Michel, G.	1984	Action of antifungal peptidolipids from Bacil-lus subtilis on the cell membrane of Saccharo-myces cerevisiae. not GLP, published The J. of Antibiotics, Vol. XXXVII, No. 2, 1984, 172-177 BMF2000-96	N	-

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[2]
AIIB-2.2	Bland, J. M., Lax, A. R. and Klich, M. A.	1990	Structure activity studies of the iturins. not GLP, published in: Peptides 1990 D. Giralt and D. Andreu (eds.) , 1990, 426-427 BMF2000-97	N	-
AIIB-2.2	Braun-Kiewnick, A. et al.	1997	Induction of systemic resistance by antagonistic Bacillus sp. and the chemical inducer benzothiadiazole controls Cercospora leaf spot of sugar beet. not GLP, published Phytopathology, Vol. 87, No. 6 (Supplement), 1997, 10 BMF2000-99	N	-
AIIB-2.1; AIIB-2.2	Butt, T.M., Harris, J.G. and Powell, K.A.	1998	"Microbial biopesticides - the European scene" (Art. 3). not GLP, published in: Methods in Biotechnology, Vol. 5 Biopesticides, Use and Delivery, 1998, 23-44 BMF2000-85	N	-
AIIB-2.1; AIIB-2.2	Eshita, S.M. et al.	1995	Bacillomycin Lc, a new antibiotic of the iturin group: isolation, structures and antifungal activities of the congeners. not GLP, published J. of Antibiotics, Vol. 48, No. 11, 1995, 1240-1247 BMF2000-84	N	-
AIIB-2.2	Gueldner, R.C.	1988	Isolation and identification of iturins as antifungal peptides in biological controls of peach brown rot with Bacillus subtilis. not GLP, published J. Agric. Food Chem., Vol. 36, 1988, 366-369 BMF2000-101	N	-

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[2]
AIIB-2.1; AIIB-2.2	Hiraoka, H., Asaka, O., Ano, T. and Shoda, M.	1992	Characterization of Bacillus subtilis RB14, coproducer of peptide antibiotics iturin A and surfactin. not GLP, published J. Gen. Appl. Microbiol., Vol. 38, 1992, 635-640 BMF2000-82	N	-
AIIB-2.2	Jacobsen, B. J. and Zidack, N.K.	2000	Induction of disease resistance mechanismus in by Bacillus subtilis strain QST713 and disease control of Erwinia caratovora subsp. betavasculorum in sugar beet. not GLP, unpublished BMF2000-94	Y	QST
AIIB-2.2	Loper J.E. and Lindow S.E.	1993	Roles of competition and antibiosis in suppression of plant diseases by bacterial biological control agents. not GLP, published Pest management: Biologically Based Technologies, 1993, 144-155 BMF2000-143	N	-
AIIB-2.1; AIIB-2.2	McKeen, C.D., Reilly, C.C. and Pusey, P.L.	1986	Production and partial characterization of antifungal substances antagonistic to Monilia fructicola from Bacillus subtilis. not GLP, published Phytopathology, Vol. 76, No. 2, 1986, 136-138 BMF2000-80	N	-
AIIB-2.1; AIIB-2.2; AIIB-2.3	Marten, P., Brückner, S. and Lüth, P.	1998	Wachstumsförderung und Biologische Kontrolle durch Bacillus subtilis Stamm B2g. not GLP, published Mitt. a.d. Biol. Bundesanst. H 357, 1998, 357 BMF2000-87	N	-
AIIB-2.1; AIIB-2.3	Schmiedeknecht, G. et al.	1998	Anwendungsmöglichkeiten von Bacillus subtilis für den Biologischen Pflanzenschutz. not GLP, published Mitt. a.d. Biol. Bundesanst. H. 357, 1998, 354 BMF2000-83	N	-

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[2]
AIIB-2.3	Stanghellini, M.E. and rasmussen, S.L.	1989	Two new diseases of Salicornia sp. caused by Bacillus subtilis and Macrophomina phaseolina. not GLP, published Phytopathology Vol. 79 (8), 1989, 912 BMF2000-150	N	-
AIIB-2.1; AIIB-2.2; AIIB-2.4; AIIB-4.2	Asaka, O., Ano, T. and Shoda, M.	1996	Persistence of Bacillus subtilis RB14 and its derivative strains in soil with respect to the LPA-14 gene. not GLP, published J. of Fermentation and Bioengineering, Vol. 81, 1996, 1-6 BMF2000-92	N	-
AIIB-2.4; AIIB-4.2; AIIB-7.1	Elsasv. J.D., Dijkstra A.F., Govaert J.M. and Veen v. J.A.	1986	Survival of Pseudomonas fluorescens and Bacillus subtilis introduced into two soils of different texture in field microplots. not GLP, published Federation of European Microbiological Societies, FEMS Microbiology Ecology 38, 1986, 151-160 BMF2000-146	N	-
AIIB-2.1; AIIB-2.2; AIIB-2.3; AIIB-2.4	Kilian, M. et al.	1998	FZB24 Bacillus subtilis - Ein Pflanzenstärkungsmittel für den kartoffelanbau. not GLP, published Mitt. a. d. Biol. Bundesanst. H. 357, No. 509a, 1998, 362 BMF2000-86	N	-
AIIB-1.3; AIIB-2.1; AIIB-2.4	Schlegel, H.G.	1985	Allgemeine Mikrobiologie. not GLP, published Georg Thieme Verlag Stuttgart New York, 1985, 70-411 BMF2000-55	N	-
AIIB-1.3; AIIB-2.4; AIIB-4.1	Slepecky, R. A.	1992	What is a Bacillus?. not GLP, published in: Biology of Bacilli (Chapter 1) Doi, R. H. and Mc Gloughlin, M. (eds.) Application to Industry Buttenworth-Heinemann, Boston, 1992, 1-21 BMF2000-58	N	-

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AIIB-1.3; AIIB-2.5; AIIB-4.1	Anonymous	1997	Project Report SC 3817 - Bacterial Characterization ATCC. not GLP, unpublished BMF2000-56	Y	QST
AIIB-2.1; AIIB-2.5; AIIIB-4.3; AIIIB-4.4	Boer de A. S. and Diderichsen, B.	1991	On the safety of Bacillus subtilis and B. amyloliquefaciens: A review. not GLP, published Appl. Microbiol. Biotechnol, Vol. 36, 1991, 1-4 BMF2000-91	N	-
AIIB-2.5	Campbell, R.	1989	Introduction to plant pathology and microbial ecology. not GLP, published in: Biological control of microbial plant pathogens, Cambridge University Press, Cambridge, Department of Botany University of Bristol (Chapter 1), 1989, 1-40 BMF2000-149	N	-
AIIB-2.2; AIIB-2.5; AIIB-4.2	Campbell, R.	1989	Biocontrol on leaf surfaces. not GLP, published in: Biological control of microbial plant pathogens Cambridge University Press, Cambridge, Department of Botany, University of Bristol Chapter 3, 1989, 66-94 BMF2000-100	N	-
AIIB-2.3; AIIB-2.5	Glick, B.R. et al.	1999	Overview of plant growth-promoting bacteria. not GLP, published in: Biochemical and Genetic Mechanisms used by plant growth promoting bacteria, (Chapter 1) Imperial College Press, Department of Biology, University of Waterloo, Ontario, Canada, 1999, 1-13 BMF2000-89	N	-

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AIIB-1.3; AIIB-2.1; AIIB-2.5	Gordon, R.E., Haynes, W.C. and Pang, C.H.-N.	1973	The Genus Bacillus. not GLP, published Agricultural Research Service United States Department of Agriculture Washington D.C. Agriculture Handbook, 427, 1973, 36-253 BMF2000-54	N	-
AIIB-2.3; AIIB-2.4; AIIB-2.5	Kararah M.A. et al.	1985	Pathophysiology in garlic gloves inoculated with Bacillus subtilis, Bacillus pumilis and Erwinia carotovora. not GLP, published Egyptian J. Phytopathology Vol. 17 (2), 1985, 131-140 BMF2000-145	N	-
AIIB-2.5	Kilian, M., Junge, H. und Krieg, U.	1998	Einfluß von Umweltfaktoren auf die ertrags-steigernde Wirkung von FZB24 Bacillus subtilis bei Kartoffeln. not GLP, published Mitt. a. d. Biol. Bundesanst., 509, 357, 1998, 361 BMF2000-153	N	-
AIIB-2.2; AIIB-2.5	Leifert, C., Chidburee S., hampson S., Workman S., Sigee D., Epton H.A.S. and Harbour A.	1995	Antibiotic production and biocontrol activity by Bacillus subtilis CL27 and Bacillus pumilus CL45. not GLP, published Journal of Applied Bacteriology, Vol. 78, 1995, 97-108 BMF2000-141	N	-
AIIB-2.2; AIIB-2.5	Loeffler W., Tschen J.S.-M., Vanittanakom N., Kugler M., Knorrp E., Hsieh T.-F. and Wu T.-g.	1986	Antifungal effects of Bacilysin and Fengmycin from Bacillus subtilis F-29-3 A comparison with activities of other Bacillus antibiotics. not GLP, published J. Phytopathology, Vol. 115 (3), 1986, 204-213 BMF2000-142	N	-

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AIIB-2.1; AIIB-2.5	Ohno, A., Ano, T. and Shoda, M.	1993	Effect of temperature change and aeration on the production of the antifungal peptide antibiotic iturin by Bacillus subtilis NB22 in liquid cultivation. not GLP, published J. of Fermentation and Bioengineering, Vol. 75, No. 1, 1993, 463-465 BMF2000-72	N	-
AIIB-2.1; AIIB-2.5	Priest, F.G.	1989	Isolation and identification of aerobic endospore-forming Bacteria. not GLP, published in: Bacillus, Chapter 3 Colin R. Harwood (ed.) The University of Newcastle upon Tyne, UK Plenum Press , 1989, 27-56 BMF2000-90	N	-
AIIB-2.1; AIIB-2.2; AIIB-2.5	Sholberg, P.L., Marchi, A. and Bechard, J.	1995	Biocontrol of postharvest diseases of apple using Bacillus spp. isolated from stored apples. not GLP, published Can. J. Microbiol. 41, 1995, 247-252 BMF2000-81	N	-
AIIB-2.1; AIIB-2.5	Sneath, P.H.A.	1986	Endospore-forming gram-positive rods and cocci. not GLP, published in: Bergey`s Manual of Systematic Bacteriology Claus, D. and Berkely, R.C.W. (eds.) The Williams & Wilkens Co., Baltimore, Vol.2, 1986, 1104-1139 BMF2000-67	N	-
AIIB-2.5	Yuan, C. and Heins, S.	2000	Longevity study of Serenade (QST 713) on pepper leaf surface in greenhouse conditions. AQ0032 not GLP, unpublished BMF2000-148	Y	QST

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AIIB-2.5	Zimmer, J., Issoufou I., Schmiede-knecht, G. und Bochow, H.	1998	Populationsdynamik, Phytoeffektivität und antagonistische Wirksamkeit von Bacillus subtilis als Nutzbakterium. not GLP, published Mitt. a.d. Biol. Bundesanst., 357, 1998, 351 BMF2000-154	N	-
AIIB-1.3; AIIB-2.1; AIIB-2.4; AIIB-2.6; AIIIB-4.4	Harwood, C. R.	1989	Introduction to the Biotechnology of Bacillus. not GLP, published Bacillus, The University of Newcastle upon Tyne, UK Plenum Press, 1989, 1-4 BMF2000-59	N	-
AIIB-1.3; AIIB-2.1; AIIB-2.3; AIIB-2.4; AIIB-2.5; AIIB-2.6; AIIB-4.2	Priest, F. G.	1993	Systematics and Ecology of Bacillus. not GLP, published in: Bacillus subtilis and other gram-positive bacteria American Society of Microbiology, Washing-ton D.C. (ed.), 1993, 3-16 BMF2000-57	N	-
AIIB-2.7	Asaka, O., To-kuda, Y., Ano, T. and Shoda, M.	1987	Plasmid instability in Bacillus subtilis during sporulation. not GLP, published Biosci. Biotech. Biochem. Vol. 19, No. 5, 1987, 639-647 BMF2000-155	N	-
AIIB-2.7	Elsas v., J.D., Govaert, J.M. and Veen v. J.A.	1987	Transfer of plasmid pFT30 between Bacilli in soil as influenced by bacterial population dy-namics and soil conditions. not GLP, published Soil Biol. Biochem. Vol. 19, No. 5, 1987, 639-647 BMF2000-156	N	-
AIIB-2.7	Ferrari, E. and Hoch, J.A.	1989	Genetics. not GLP, published in: Bacillus, Colin R. Harwood (ed), The University of Newcastle upon Tyne New-castle upon Tyne, UK, Plenum Press, 1989, 57-72 BMF2000-157	N	-

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AIIB-2.7	Glick, B.R., Patten, C.L., Holguin, G. and Penrose, D.M.	1999	Deliberate environmental release of bacteria. not GLP, published in: Biochemical and Genetic Mechanisms used by plant growth promoting bacteria, Imperial College Press, Department of Biology, University of Waterloo, Ontario, Canada (Chapter 8), 1999, 249-267 BMF2000-158	N	-
AIIB-2.7	Graham, J.B. and Istock, C.A.	1979	Gene exchange and natural selection cause Bacillus subtilis to evolve in soil culture. not GLP, published Science Vol. 204, 1979, 637-639 BMF2000-159	N	-
AIIB-2.7	Hemilä, H., Glode, L.M. and Palva, I.	1989	Production of diphteria toxin CRM228 in B. subtilis. not GLP, published Fed. Eur. Micrbiol. Soc. Lett., Vol. 65, 1989, 193-198 BMF2000-160	N	-
AIIB-2.7	Klier, A., Bourgouin, C. and Rapoport, G.	1983	Mating between Bacillus subtilis and Bacillus thuringiensis and transfer of cloned crystal genes. not GLP, published Mol. Gen. Gent., Vol. 191, 1983, 257-262 BMF2000-161	N	-
AIIB-2.7	Leonhardt, H. and Alonso, J.C.	1991	Parameters affecting plasmid stability in Bacillus subtilis. not GLP, published Gene, Vol. 103, 1991, 107-111 BMF2000-162	N	-
AIIB-2.7	Mountain, A.	1989	Gene expression systems for Bacillus subtilis. not GLP, published in: Bacillus, Colin R. Harwood (ed) The University of Newcastle upon Tyne Newcastle Tyne, UK, Plenum Press (Chapter 5), 1989, 73-114 BMF2000-163	N	-

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AIIB-2.7	Ohno, A., Ano, T. and Shoda, M.	1995	Production of a lipopeptide antibiotic surfactin, by recombinant Bacillus subtilis in solid state fermentation. not GLP, published Biotechnology and Bioengineering, Vol. 47 (2), 1995, 209-214 BMF2000-164	N	-
AIIB-2.8	Bar, J.G.	1975	CHANGES IN THE EXTRACELLULAR ACCUMULATION OF ANTIBIOTICS DURING GROWTH AND SPORULATION OF BACILLUS SUBTILIS IN LIQUID CULTURE:. not GLP, published J. appl. Bact., Vol 39, 1975, 1-13 BMF2000-173	N	-
AIIB-2.2; AIIB-2.8	Besson, F., Peypoux, F. and Michel, G.	1979	Antifungal activity upon Saccharomyces cerevisiae of Iturin A, Mycosubtilin, Bacillomycinl and of their derivates; inhibition of this antifungal activity by lipid antagonists. not GLP, published The J. of Antibiotics, Vol. XXXII, No. 8, 1979, 828-833 BMF2000-95	N	-
AIIB-2.8	Besson, F., Peypoux, F, Michel, G. and Delcambe, L.	1976	CHARACTERIZATION OF ITURIN A IN ANTIBIOTICS FROM VARIOUS STRAINS OF BACILLUS SUBTILIS:. not GLP, published The Journal of Antibiotics, Vol. XXIX, 10, 1976, 1043-1049 BMF2000-174	N	-
AIIB-2.2; AIIB-2.8	Bland, J. M., Lax, A.R. and Klich, M.A.	1995	Iturin-A, a antifungal peptide produced by Bacillus subtilis. not GLP, published Pre. Plant Growth Regent Soc. Am. Vol. 22, 1995, 102-107 BMF2000-98	N	-

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AIIB-2.1; AIIB-2.2; AIIB-2.3; AIIB-2.8	Citernesi, A.S., Filippi, C., Bagnoli, G. and Giovannetti, M.	1994	Effects of the antimycotic iturin A2, secreted by Bacillus subtilis strain M51, on arbuscular mycorrhizal fungi. not GLP, published Microbiological research, Vol. 149, 1994, 241-246 BMF2000-71	N	-
AIIB-2.8	Delcambe, L. et al.	1977	STRUCTURE OF ITURIN AND ITURIN-LIKE SUBSTANCES:. not GLP, published Biochemical Society Transactions, Vol 5, 569th Meeting, Sussex, 1977, 1122-1124 BMF2000-175	N	-
AIIB-2.8	Feignier, C., Besson, F. and Michel, G.	1995	STUDIES ON LIPOPEPTIDE BIOSYNTHESIS BY BACILLUS SUBTILIS: ISOLATION AND CHARACTERIZATION OF ITURIN-; SURFACTIN+ MUTANTS:. not GLP, published FEMS Microbiology Letters 127, 1995, 11-15 BMF2000-176	N	-
AIIB-2.8	Katz, E. and Demain, A.L.	1977	THE PEPTIDE ANTIBIOTICS OF BACILLUS: CHEMISTRY; BIOGENESIS; AND POSSIBLE FUNCTIONS. not GLP, published Bacteriological Reviews, 1977, 449-474 BMF2000-177	N	-
AIIB-2.2; AIIB-2.8	Klich M.A., Lax A.R. and Bland J.M.	1991	Inhibition of some mycotoxigenic fungi by iturin A, a peptidolipid produced by Bacillus subtilis. not GLP, published Mycopathologia, Vol. 116, 1991, 77-80 BMF2000-140	N	-

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AIIB-2.8	Latoud, C., Peypoux, F. and Michel, G	1987	ACTION OF ITURIN A, AN ANTIFUNGAL ANTIBIOTIC FROM BACILLUS SUBTILIS; ON THE YEAST SACCHAROMYCES CE-REVISIAE: MODIFICATIONS OF MEMB-RANE PERMEABILITY AND LIPID COM-POSITION. not GLP, published The Journal of Antibiotics, Vol. XL, 11, 1987, 1588-1595 BMF2000-178	N	-
AIIB-2.1; AIIB-2.5; AIIB-2.8	Priest, F.G.	1989	Products and Applications. not GLP, published in: Bacillus, Colin R. Harwood (ed.) The University of Newcastle upon Tyne, UK, Plenum Press, Chapter 11, 1989, 293-320 BMF2000-69	N	-
AIIB-2.2; AIIB-2.8	Thimon L., Maget-Dana R. and Michel G.	1992	Surface active properties of antifungal lipopep-tides produced by Bacilluis subtilis. not GLP, published JAOCS, Vol. 69 (1), 1992, 92-93 BMF2000-144	N	-
AIIB-2.1; AIIB-2.8	Zimmermann, S.B. et al.	1987	Difficidin and Oxydifficidin: Novel broad spectrum antibacterial antibiotics produced by Bacillus subtilis. not GLP, published J. Antibiotics, Vol. 40 (12), 1987, 1677-1681 BMF2000-70	N	-
AIIB-2.1; AIIB-2.5; AIIB-2.8; AIIB-2.9; AIIB-3.7; AIIIB-4.3; AIIIB-4.4	EPA	1997	Final Decision Document, TSCA Section 5 (H) (4) Exemption for Bacillus subtilis. not GLP, published EPA, 1997 BMF2000-93	N	-
AIIB-2.5; AIIB-2.9	Fossum, K., Herikstad, H., Binde, M. and Pettersen K.-E.	1986	Isolations of Bacillus subtilis in connection with Bovine Mastitis. not GLP, published Nordisk Veterinärmedicine, Vol. 38, 1986, 233-236 BMF2000-151	N	-

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AIIB-2.9	Harwood, C.R. (ed.)	1989	BACILLUS. not GLP, published The University of Newcastle upon Tyne, UK, Plenum Press (UPDATED LINKAGE MAP OF B: SUBTILIS in Annex X, 1989, 363-406 BMF2000-179	N	-
AIIB-2.5; AIIB-2.9	Ihde, D.C. and Armstrong D.	1973	Clinical spectrum of infection due to bacillus species. not GLP, published Amer. J. Med., Vol. 55, 1973, 839-845 BMF2000-152	N	-
AIIB-2.1; AIIB-2.7; AIIB-2.8; AIIB-2.9	Matsuno, C.D., Hitomi, T., Ano, T. and Shoda M.	1992	Transformation of Bacillus subtilis, antifungal-antibiotic iturin producers with isolated antibiotic resistance plasmids. not GLP, published J. Gen. Appl. Microbiol., Vol. 38, 1992, 13-21 BMF2000-77	N	-
AIIB-2.1; AIIB-2.2; AIIB-2.5; AIIB-2.9	Phae, C.-G., Sasaki, M., Shoda, M. and Kubota, H.	1990	Characteristics of Bacillus subtilis isolated from composts suppressing phytopathogenic microorganisms. not GLP, published Soil Sci. Plant Nutr., Vol. 36(4), 1990, 575-586 BMF2000-74	N	-
AIIB-2.7; AIIB-2.9	Saris, P., Taira, S., Airaksinen, U., Palva A., Sarvas, M., Palva I. and Runeberg-Nyman, K.	1990	Production and secretion of pertussis toxin subunits in Bacillus subtilis. not GLP, published FEMS Microbiology Letters, Vol. 68, 1990, 143-148 BMF2000-166	N	-
AIIB-2.7; AIIB-2.9	Saris, P.E.J., Airaksinen, U., Nurmiharju, S., Runeberg-Nyman, K. and Palva I.	1990	Expression of Bordetella pertussis toxin subunits in Bacillus subtilis. not GLP, published Biotechnology Letters, Vol. 12 (12), 1990, 873-878 BMF2000-165	N	-

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AIIB-2.7; AIIB-2.9	Taira, S., Jalonen, E., Paton, J.C., Sarvas M. and Runeberg-Nyman, K.	1989	Production of pneumolysin, a pneumococcal toxin in Bacillus subtilis. not GLP, published Gene, Vol. 77, 1989, 211-218 BMF2000-167	N	-
AIIB-2.7; AIIB-2.9	Tokuda, Y., Ano, T. and Makoto, S.	1993	Characterictics of plasmid stability in Bacillus subtilis NB22, an antifungal antibiotic iturin producer. not GLP, published J. of Fermentation and Bioengineering, Vol. 75 (4), 1993, 319-321 BMF2000-168	N	-
AIIIA-2.2	Anonymous	2000	Minimum Explosive Concentration. K000726B.FMR not GLP, unpublished PHY2001-128	Y	QST
AIIIA-2.3	Anonymous	2001	Hintergrund Information zur Entflammbarkeit von Stäuben. not GLP, unpublished PHY2001-129	Y	QST
AIIIA-2.6	Cunningham Hilbig, Laura	2000	Liquid density test method using a pycnometer. FC4402 not GLP, unpublished PHY2001-131	Y	QST
AIIIA-2.6	Ryder Fox, Jennifer	2000	Bulk density Test Method for Powders & Granules. FC4401 not GLP, unpublished PHY2001-130	Y	QST
AIIIA-2.8.1	Cunningham Hilbig, Laura	2000	Wet time method for powders & granules. FC4404 not GLP, unpublished PHY2001-132	Y	QST
AIIIA-2.8.3	Cunningham Hilbig, Laura Ryder Fox, Jennifer	2000	Suspensibility & Resuspensibility determination for dry products. FC4405 not GLP, unpublished PHY2001-133	Y	QST

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AIIIA-2.8.5	Cunningham Hilbig, Laura Ryder Fox, Jennifer	2000	Wet sieve test method for powders & granules. FC4406 not GLP, unpublished PHY2001-134	Y	QST
AIIIA-2.8.1; AIIIA-2.8.2; AIIIA-2.8.3; AIIIA-2.8.6	Anonymous	1999	Serenade WP Physical Property Analysis Summary. not GLP, unpublished PHY2001-65	Y	QST
AIIIB-2.1	Anonymous	2000	Physical/Chemical Properties. not GLP, unpublished PHY2000-720	N	QST
AIIIB-2.2; AIIIB-4.4; AIIIB-5.1	Gingras, B. A.	1999	Storage Stability of Qst 713 Strain of Dried Bacillus subtilis with-Residual Fermentation media Identified as Qst 713 WP. Project No. L08726 SN9 GLP, unpublished PHY2000-721	Y	QST
AIIIB-2.1; AIIIB-2.2; AIIIB-2.4.1; AIIIB-2.4.2; AIIIB-2.4.4; AIIIB-2.4.5	Walter, Dieter	2000	Physico-chemical Properties of the Formulati-on QST 713 WP after Accelerated Storage at 40°C for 8 Weeks. 99430/01-PCAS GLP, unpublished PHY2000-722	Y	QST
AIIIB-2.6; AIIIB-4.4	Campbell, B., Cunningham-Hilbig, L.	2000	Serenade WP Tank Mix compability summary Report. not GLP, unpublished PHY2000-726	Y	QST
AIIIB-2.6; AIIIB-4.4; AIIIB-7.5	Campbell, B., Cunningham-Hilbig, L.	2000	Serenade WP Tank Mix compability interim Report. not GLP, unpublished PHY2000-725	Y	QST
AIIIB-2.6	Gingras, B. A.	2000	Serenade WP Tank Mix compability interim Report. not GLP, unpublished PHY2000-727	Y	QST

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[2]
AIIIB-2.6	Walter, D.	2000	Surface Tension of QST 713 WP. Study code: 99430/01-PCST GLP, unpublished PHY2000-723	N	QST

Codes of owner

QST: AgraQuest, Inc.

Annex B

Bacillus subtilis strain QST 713

B-3: Data on application and further data

B.3 Data on application and further data

B.3.1 Further information on the micro-organism (Annex IIB 3)

B.3.1.1 Function (Annex IIB 3.1)

Biocontrol agent with fungicidal and bactericidal action.

B.3.1.2 Field of use envisaged (Annex IIB 3.2)

Serenade™ WP is envisaged to be used in open fields and under protected cultivation in several horticultural crops and in viticulture.

B.3.1.3 Crops or products protected or treated (Annex IIB 3.3)

The intended uses of Serenade™ WP envisaged are in apple, pear, stone fruit and viticulture in open fields and in lettuce under both open field conditions and protected cultivation.

B.3.1.4 Method of production and quality control (Annex IIB 3.4)

B.3.1.4.1 Composition of QST 713 Technical

QST 713 Technical contains 146 g/ kg of the active agent (dried *B. subtilis* 5 x 10^{10} cfu/g) and 854 g/ kg insoluble or soluble residues of the fermentation process.

B.3.1.4.2 Description of the production process

Confidential information about the details, see Annex C.

B.3.1.4.3 Quality and purity control

Confidential information, see Annex C.

B.3.1.4.4 Techniques used to ensure a uniform product/ assay methods for standardisation

A uniform product is achieved by employing a standard fermentation process, carefully performing all process steps, and by applying quality control.
Quality control analysis is performed on every Lot of QST 713 Technical to ensure a consistent product.

Confidential information about the details, see Annex C.

B.3.1.5 Information on the occurrence or possible occurrence of the development of resistance of the target organism(s) (Annex IIB 3.5)

There is a single report in the literature describing development of resistance against *B. subtilis*. In glass house trials with Astilbe micro-plants under condition which favour fungal attack it was found that resistance in the fungal pathogen *Botryotinia fuckeliana* developed within 10 consecutive crop generations. Results of *in vitro* assays suggested that the fungal strains had become resistant to two antibiotics known to be the principle control mechanism

of the *B. subtilis* isolate (Leifert, 1994). In the case of the bacterial strain QST 713 in Serenade™ WP the mode of action of strain QST 713 of *Bacillus subtilis* has been demonstrated to rely on a broader base than single site action, since it includes diverse mechanisms not easily overcome by pathogens. Further, up to now there is no indication of decreasing efficacy of the *Bacillus subtilis* strain in Serenade™ WP (QST 713) against fungal pathogens to be controlled. The risk of development of resistance is therefore classified as low.

B.3.1.6 Methods to prevent loss of virulence of seed stock of the micro-organism (Annex IIB 3.6)

AgraQuest Inc. takes precautions in order to guarantee purity and to prevent loss of virulence of QST 713 strain of *B. subtilis* (Bellet 1998, BMF 2000-66)

Confidential information about the details, see Annex C.

B.3.1.7 Recommended methods and precautions concerning handling, storage, transport or fire (Annex IIB 3.7)

Frommer et al. (1989) relate the different risk classes according to European classification of micro-organisms to adequate safety precautions for biotechnology. In this review *B. subtilis* is not even listed as a pathogen but mentioned as a harmless micro-organism (among numerous others) with a long history of safe use in enzyme production. The authors state that these micro-organisms do not require a containment to protect workers, just the defined measures of GILSP (Good Industrial Large Scale Practise). *B. subtilis* falls under the Class 1 Containment of European Federal Law of Biotechnology and also is considered a Class 1 Containment Agent by the US National Institute of Health (NIH) (EPA, 1997).

The producer, AgraQuest Inc., states that at the manufacturing facilities worker exposure is minimised due to rigorous application of Good Manufacturing Practise (GMP) and quality control.
Evaluating the acute toxicity studies the State of California, Department of Pesticide Regulation, concludes that no precautionary statement on the product label for the acute dermal toxicity hazard are required (Duncan and Leung, 1999: Product Registration Recommendation Sheet). The applicant therefore merely recommends general protection measures in **handling**, such as:

- To wash hands and exposed skin before eating, drinking or smoking after handling the product.
- To wash any contamination from skin and eye immediately with soap and water or water, respectively and to drink plenty of water if swallowed.
- To avoid direct contact.

Further precautions concerning **storage** and disposal are:

- To keep product in original container, tightly closed in a safe place, locked from children
- Not to store the product together with food or feeding stuff
- To keep product away from waters
- To thoroughly rinse empty containers with water and to add empty packages to local waste disposal or recycling system

According to the storage stability report QST 713 Technical can be stored under warehouse conditions at ambient temperature without loss of stability over a one year period in minimum (Gingras, 1999). In conclusion, storage of QST 713 Technical does not require specific conditions to ensure stability.

Transport of QST 713 Technical does not require special precautions or restrictions due to its non-reactive, non-corrosive and non-flammable character and its general safety concerning human health and the environment.

Since QST 713 Technical is not flammable there is no need for special **fire** precautions or fire fighting procedures.

B.3.1.8 Procedures for destruction or decontamination (Annex IIB 3.8)

With regard to destruction and decontamination the applicant gives the following general information and recommendations and measures in case of accidental release:

- **Decomposition is achieved by thermal combustion at 600 °C without any residues or hazardous products.**
- To keep product away from waters
- To empty container completely, rinse thoroughly and dispose of safely
- To dispose of product in accordance with all applicable federal, state, and local environmental regulations.

With regard to the fermentation process yielding QST 713 Technical it is stated that in case contaminant micro-organisms are being detected at a potentially hazardous level the affected batch will be treated adequately and analysed again to prove control of contaminants.

Confidential information about the details, see Annex C.

B.3.1.9 Measures in case of an accident (Annex IIB 3.9)

In case direct contact to *B. subtilis* QST 713 material occurs the applicant recommends to follow general first aid instructions:

- If inhaled: move to fresh air
- If in contact with eyes: flush eyes with plenty of water
- If in contact with skin: wash skin with soap and water
- If swallowed: give large amounts of water

The applied protection measures for workers will adequately serve in case material of QST 713 Technical is released or spilled accidentally.
The contaminated area may be cleaned by sweeping up spill and safely disposing of in accordance with all applicable federal, state, and local environmental regulations.

B.3.2 Data on application of the preparation (Annex IIIB 3)

B.3.2.1 Field of use envisaged (Annex IIIB 3.1)

Serenade™ WP is envisaged to be used in fields and under protected cultivation in several horticultural crops and in viticulture.

B.3.2.2 Mode of action (Annex IIIB 3.2)

Nature of the effects on harmful organisms:

Fungistatic, fungicidal, bactericidal, indirectly via induction of resistance in the host plant.

Serenade™ WP is acting fungistatic and fungitoxic by disruption of hyphae following contact with the fungal pathogen on the leaf surface. High efficacy of this plant protection product is provided when the *B. subtilis* cells colonize the leaf surface to form a protective layer before fungal attack has occurred. Besides antibiosis nutrient competition is involved in the mode of action and more importantly *B. subtilis* induces a systemic resistance response of the plant, indicated by enhanced peroxidase production (Jacobsen and Zidack, 2000). This mechanism also is relevant for the proved activity against the bacterial infection fire blight caused by *Erwinia amylovora*.

B.3.2.3 Details of intended use (Annex IIIB 3.3)

The intended uses of Serenade™ WP envisaged are in viticulture, pome fruit, stone fruit in the field and lettuce under field conditions and protected cultivation as well. The application rates per treatment for orchards is 5-15 kg/ha, in viticulture and lettuce 4-12 kg/ha. The maximum numbers of applications are 16 in pome fruit against scab, but only 4 against fire blight. In stone fruit 10 and in viticulture 8 numbers of applications are envisaged. In the case of lettuce there are no number of applications given because after planting the fungicide agent should be sprayed in an interval of 5 to 7 days up to the day of harvest. In all cases the application method is spraying at infestation or according to local extension service. In the case of the control of fire blight this time of application is during blossom. For further details see list of uses below.

List of uses supported by available data

Crop and/ or situation (a)	Member State or Country	Product name	F G or I (b)	Pests or Group of pests controlled (c)	Formulation		Application			Product application rate per treatment (g)			PHI (days) (h)	Remarks (i)
					Type (d)	Conc. of as (e)	method kind	growth stage (f)	number min max	kg/100 L	water l/ha min max	kg/ha min max		
Orchards, Apple, Pear	North- and South-Europe	Serenade™ WP	F	*Venturia inaequalis* (scab) *Erwinia amylovora* (fire blight)	WP	5 x 10^9 cfu/g	spraying	BBCH 10 to 72 during blossom	1 - 16 4	1 (i.e. 10^{12} cfu)	500 - 1.500	5 - 15	---	*
Orchards, stone fruits		Serenade™ WP	F	*Monilia*	WP	5 x 10^9 cfu/g	spraying	BBCH 55 to 69 BBCH 70 to 84 BBCH 85 to 89	4 4 2	1 (i.e. 10^{12} cfu)	500 - 1.500	5 - 15	---	* **
Grapevines	Middle- and South-Europe	Serenade™ WP	F	*Uncinula necator (Oidium)* *Botrytis cinerea*	WP	5 x 10^9 cfu/g	spraying	BBCH 55 to 75 BBCH 68 to 81	1 - 8 1 - 4	1 (i.e. 10^{12} cfu)	400 - 1.200	4 - 12	---	*
Lettuce	North- and South-Europe	Serenade™ WP	F, G	*Bremia lactucae*	WP	5 x 10^9 cfu/g	spraying	after planting	--	1 (i.e. 10^{12} cfu)	400 – 1.200	4 - 12	---	**

(a) EU and Codex classification
(b) Outdoor or field use (F), glasshouse application (G) or indoor application (I)
(d) internationally (GIFAP) agreed codes
(e) cfu = colony forming units
(f) according to BBCH scale; grapevines: 55 inflorescences swelling, flowers closely pressed together; 75: berries pea-sized, bunches hang; 68: 80% of flowerhoods fallen; 81: beginning of ripening: berries begin to develop variety- specific colour;
Application timing: exact timing depends on local conditions: spray when infestation will occur or according to local extension service
(g) minimum pre-harvest interval not relevant, no residues
(h) product stated as active substance expressed in colony forming units (cfu)
(i) * spray interval max. 5 days; use product in spraying sequence with other fungicides
** spray interval 5 to 7 days, up to the day of harvest

B.3.2.4 Application rate (Annex IIIB 3.4)

The application rate per treatment for orchards is 5-15 kg/ha, in viticulture and lettuce 4-12 kg/ha.

B.3.2.5 Content of micro-organism in material used (diluted spray) (Annex IIIB 3.5)

For all fields of application the spray is a 1 % dispersion of Serenade WP (10 g product/l), containing 10 % active substance on average. The resulting concentration of active ingredient in the spray is 1 g/l.

B.3.2.6 Method of application (Annex IIIB 3.6)

In all cases the application method is spraying.

B.3.2.7 Number and timing of applications and duration of protection (Annex IIIB 3.7)

Time of application is at infestation or according to the local extension service. In the case of the control of fire blight the time of application is during blossom. In the case of lettuce the fungicide agent should be sprayed in an interval of 5 to 7 days up to the day of harvest. The maximum number of applications is 16 in apple and pears against scab, but only 4 against fire blight. In stone fruit 10 and in viticulture 8 applications are envisaged.

B.3.2.8 Necessary waiting periods or other precautions to avoid phytopathogenic effects on succeeding crops (Annex IIIB 3.8)

It is to assume that there are no adverse effects on succeeding crops by the use of the product Serenade^{TM} WP.

B.3.2.9 Proposed instructions for use (Annex IIIB 3.9)

No special comments are necessary.

B.3.3 Further information on the plant protection product (Annex IIIB 4)

B.3.3.1 Packaging and compatibility of the preparation with proposed packaging materials (Annex IIIB 4.1)

Its physical and toxicological properties, characterise Serenade^{TM} WP as non-reactive and non-hazardous. The solid, inert product does not require special stability or resistance properties of the packaging or the material used in packaging.

Packaging material: 10 kg paper bag with high density polyethylene inlet (0.0005 HDPE), three different quality kraft paper layers comprising the outer envelope (from outside: 1st layer: white 50 lb kraft paper, 2nd: brown 40 lb kraft paper, 3rd: brown 50 lb kraft paper).
Size: 33 x 6.4 x 61 cm, unfilled
Opening: 30 cm diameter, open mouth

Closure: gussetted bag (gussett = folding side panel), glue is applied to mouth opening and heat sealed

B.3.3.2 Procedures for cleaning application equipment (Annex IIIB 4.2)

Serenade™ WP is dispensable in water and can be removed from surfaces with water. Apart from gloves there is no requirement for wearing protective clothing. Following contact it is recommended to wash hands with soap and water.

Cleaning procedure: rinse application equipment thoroughly with water.

B.3.3.3 Re-entry periods, necessary waiting periods or other precautions to protect man, livestock and the environment (Annex IIIB 4.3)

In principle, Serenade™ WP does not pose residue problems since the active agent, *B. subtilis*, is a frequent, ubiquitous micro-organism in the soil.
The sole factor of concern is the optical appearance of harvested crop: if Serenade™ WP is applied right before harvest the appearance of fruits may be impaired by a white spray cover. Considering this it is recommended not to use Serenade™ WP later than the mentioned maximum growth stages (see Vol. 1, endpoints). There is no need for pre-harvest or re-entry intervals given since remaining *B. subtilis* cells do not present a health or environmental risk:

- Toxicity and ecotoxicity testing proved that any adverse or hazardous effects arising from application of Serenade™ WP can be excluded.
- The EPA (1997) states that the only human health concern for (the more intensely exposed) workers in the fermentation facility is the potential of *B. subtilis* to elicit allergic reactions in individuals repeatedly exposed to subtilisin (a proteinaceous compound produced by *B. subtilis*). This risk is minimised by appropriate limits set by the U.S. OSHA (Occupational Safety and Health Administration) for subtilisin in the industrial setting, and by protective clothing worn by workers.
- *B. subtilis* is characterized as non-pathogenic in the literature (Boer and Diderichsen, 1991). It falls under Class 1 Containment of European Federal Law of Biotechnology and is considered a Class 1 Containment Agent by the U.S. National Institute of Health (EPA, 1997). *B. subtilis* does not require containment to protect workers since it is a harmless micro-organism with a long history of safe use in enzyme production (Frommer et al., 1989).
- The global distribution and predominance of *B. subtilis* surpasses any residual cells occurring after harvest of crop products and these residual cells may easily be removed by water.
- Proliferation of remaining *B. subtilis* cells during processing of raw products (grapes, apples to vine, juice respectively) is not relevant since a) in vine fermentation conditions are unfavourable and b) in juice production microbial contaminants are heat killed (at a processing temperature of ~ 90 °C), while conditions do not favour endospore formation.

Consequently, application of Serenade™ WP does not necessitate pre-harvest and re-entry intervals or a withholding period for animal feeding-stuff.

Information on any specific agricultural, plant health or environmental conditions under which the preparation may or may not be used

None of the test results obtained or observations made were such that restrictions should be imposed.

B.3.3.4 Recommended methods and precautions concerning: handling, storage, transport or fire (Annex IIIB 4.4)

For Serenade™ WP there are no requirements for classification and labelling, as proved by the above given physical characteristics, and by the submitted toxicological and ecotoxicological data.
Only general precautions are recommended to be applied in handling and storage of the undiluted product. This information is presented under the form of a safety data sheet pursuant to Council Directive 67/548/EEC and an Instruction Leaflet (Safety Data Sheet, PHY2000-69, and Instruction Leaflet, PHY2000-71):

- To avoid contact with skin
- To wash any contamination from eyes or skin immediately
- To wash exposed hands before consuming food, drinking or smoking
- To store the product in the original container, in a dry area inaccessible to children
- To completely empty the bag into the application equipment, rinse it with water and safely dispose of the clean bag
- To wash application equipment and spray tank with water after use (no specific cleaning agents)
- To keep the product away from waters

Serenade™ WP can be mixed with chemical or other biological plant protection products or chemical substances provided that experience has shown physical compatibility and efficacy of the combination. Studies on Serenade™ WP tank mix compatibility with several biological and chemical plant protection products indicated physical compatibility and unaltered efficacy of Serenade™ WP (Campell and Cunningham Hilbig 2000, PHY2000-725; Campell and Cunningham Hilbig 2000, PHY2000-726). Mixtures with substances attacking organic material should be avoided.

According to the **storage** stability report of QST 713 WP this product remains stable for at least one year of storage at ambient temperature under warehouse conditions, thus no special requirements are necessary (Gingras, 1999). According to the producer, who monitored the product over several years, a minimum storage stability of 2 years is realistic.

Transport of Serenade™ WP does not require special precautions since neither the ingredients nor the formulated end product have corrosive, reactive or flammable properties, and the active agent, *B. subtilis*, is generally regarded as safe (Harwood 1989, BMF 2000-59; EPA, 1997; Boer and Diderichsen, 1991).

Since Serenade™ WP is not flammable there is no need for special **fire** precautions or fire fighting procedures. All regular fire extinguishing media can be employed: water mist, foam, carbon dioxide and dry powder.

Statement of the risks arising and the recommended methods to minimise those risks:
No specific risks are related to handling, storage or transport of Serenade™ WP. Handling of the undiluted product should be done wearing universal protective gloves (plant protection) to

avoid skin contact. Besides this general preventive measure, no protective clothing or equipment is required. No special fire fighting procedures are to be employed. As a general precaution measure Serenade™ WP shall be kept away from waters.

B.3.3.5 Measures in the case of an accident (Annex IIIB 4.5)

An accidental release of product indoor or outdoor does not require special treatment. Following accidental inhalation, ingestion or skin and eye contact no specific treatment is necessary.
In case direct contact occurred the submitted Safety Data Sheet gives following instructions:

- if inhaled: move exposed person to fresh air
- if in contact with eyes: flush eyes with plenty of water
- if in contact with skin: wash with soap and water
- if swallowed: drink plenty of water

No specific treatment after contact with the product is recommended since no specific symptoms are known to occur.

B.3.3.6 Procedures for destruction or decontamination of the plant protection product and its packaging (Annex IIIB 4.6)

According to the Safety Data Sheet emptied containers should be rinsed thoroughly with water prior to disposal or recycling. The disposal of product has to be performed in accordance with all applicable federal, state and local environmental regulations.
Wastes resulting from the use of Serenade™ WP, i.e. residual water dispersions, may be disposed of on site or at an approved waste disposal facility.
The same procedure is applicable to larger quantities, which may occur very rarely only since the spray is mixed immediately before use and is intended to be used up by application.
Complete decomposition of solid waste is achieved by thermal combustion at 600 °C without creating any residues or hazardous products.

B.3.4 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[3]

[3] Only notifier listed

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[3]
AIIB-3.5	Li, H. and Leifert, C	1994	DEVELOPMENT OF RESISTANCE IN BOTRYOTINIA FUCKELIANA (DE BARY) WHETZEL AGAINST THE BIOLOGICAL CONTROL AGENT BACILLUS SUBTILIS CL27. not GLP,published Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz, Vol. 101, 1994, 414-418 BMF2000-180	N	-
AIIB-3.5	Roush, R.T.	1998	STRATEGIES FOR RESISTANCE MANAGEMENT (Art. 30). not GLP,published Methods in Biotechnology, Vol. 5: Biopesticides: Uses and Delivery, 1998, 575-593 BMF2000-181	N	-
AIIB-3.7	Anonymous	1998	BPPD COMPANY FEDERAL REGISTER DOCUMENT SUBMISSION TEMPLATE (12/1/98). not GLP, unpublished BMF2000-182	Y	QST
AIIB-3.7	Duncan, R. A. and Leung, P.	1999	PRODUCT REGISTRATION RECOMMENDATION SHEET. not GLP, unpublished BMF2000-183	Y	QST
AIIB-3.7; AIIIB-4.3	Frommer, W. et al.	1989	SAFE BIOTECHNOLOGY III. SAFETY PRECAUTIONS FOR HANDLING MICROORGANISMS OF DIFFERENT CLASSES. not GLP,published Appl. Microbiol. Biotechnol., Vol. 30, 1989, 541-552 BMF2000-184	N	-
AIIIB-3.9; AIIIB-4.4	Anonymous	2000	Serenade WP Instrucions for Use. not GLP, unpublished PHY2001-71	N	QST
AIIIB-4.4; AIIIB-12.3	Anonymous	2000	Safety Data Sheet: Serenade WP. not GLP, unpublished PHY2001-69	N	QST

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[3]
AIIB-2.1; AIIB-2.5; AIIIB-4.3; AIIIB-4.4	Boer de A. S. and Diderich-sen, B.	1991	On the safety of Bacillus subtilis and B. amy-loliquefaciens: A review. not GLP,published Appl. Microbiol. Biotechnol, Vol. 36, 1991, 1-4 BMF2000-91	N	-
AIIIB-2.6; AIIIB-4.4	Campbell, B., Cunningham-Hilbig, L.	2000	Serenade WP Tank Mix compability summary Report. not GLP, unpublished PHY2000-726	Y	QST
AIIIB-2.6; AIIIB-4.4; AIIIB-7.5	Campbell, B., Cunningham-Hilbig, L.	2000	Serenade WP Tank Mix compability interim Report. not GLP, unpublished PHY2000-725	Y	QST
AIIB-2.1; AIIB-2.5; AIIB-2.8; AIIB-2.9; AIIB-3.7; AIIIB-4.3; AIIIB-4.4	EPA	1997	Final Decision Document, TSCA Section 5 (H) (4) Exemption for Bacillus subtilis. not GLP,published EPA, 1997 BMF2000-93	N	-
AIIIB-2.2; AIIIB-4.4; AIIIB-5.1	Gingras, B. A.	1999	Storage Stability of Qst 713 Strain of Dried Bacillus subtilis with-Residual Fermentation media Identified as Qst 713 WP. Project No. L08726 SN9 GLP, unpublished PHY2000-721	Y	QST
AIIB-1.3; AIIB-2.1; AIIB-2.4; AIIB-2.6; AIIIB-4.4	Harwood, C. R.	1989	Introduction to the Biotechnology of Bacillus. not GLP,published Bacillus, The University of Newcastle upon Tyne, UK Plenum Press, 1989, 1-4 BMF2000-59	N	-

Codes of owner

QST: AgraQuest, Inc.

Annex B

Bacillus subtilis strain QST 713

B-4: Proposals for the classification and labelling

B.4 Proposals for the classification and labelling

B.4.1 Proposals including justification of the proposals for the classification and labelling of the active substance in accordance with directive 67/548/EEC

With regard to environmental fate and behaviour no EU-criteria for classification and labelling of micro-organisms are available.

The following is proposed in accordance with the latest classification and labelling guidance under Directive 67/548/EEC (i.e. in the 18th ATP published as Directive 93/21/EEC):

***Bacillus subtilis* (strain QST 713)**

Hazard symbol:	Xi	
Indication of danger:	Irritant	
Risk phrases:	R 43	May cause sensitisation by skin contact
Reasons for classification		
For justification of R 43 see	B.6.1.1.6	Absence of any data on skin sensitisation by the active ingredient
and		
	B.6.7.3	Skin sensitisation of the formulation

B.4.2 Proposals including justification of the proposals for the classification and labelling of the preparation in accordance with directive 67/548/EEC

The following is proposed in accordance with Directive 78/631/EEC in combination with the latest classification and labelling guidance under Directive 67/548/EEC (i.e. in the 18th ATP published as Directive 93/21/EEC):

QST 713 WP (Serenade™ WP)

Hazard symbol:	Xi	
Indication of danger:	Irritant	
Risk phrases:	R 43	May cause sensitisation by skin contact
Reasons for classification		
For justification of R 43 see	B.6.7.3	Skin sensitisation

B.4.3 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[4]
AIIIB-4.4; AIIIB-12.3	Anonymous	2000	Safety Data Sheet: Serenade WP. not GLP, unpublished PHY2001-69	N	QST

Codes of owner

QST: AgraQuest, Inc.

[4] Only notifier listed

Annex B

Bacillus subtilis strain QST 713

B-5: Methods of analysis

B.5 Methods of analysis

B.5.1 Analytical methods for formulation analysis (Annex IIB 4.1; Annex IIIB 5.1)

B.5.1.1 Methods for the identification of the micro-organism (Annex IIB 4.1)

General statement on analytical methods

In principle *B. subtilis* is identified and analysed using biological methods, i.e. plating on microbiological media. The central criterion for identification of *Bacillus* species is the endospore morphology. For identification of different strains additional criteria are microscopic appearance, colony morphology and physiological characteristics. During fermentation and production processes the immediate microscopic appearance of a drop of liquid culture is continuously checked for purity.
In addition, cultures on agar used for microscopical and macroscopical identification of extraneous micro-organisms.

Definite identification of strain QST 713 as *Bacillus subtilis* is achieved by applying following parameters:

- Presence of single, gram-positive rods with peritrichous flagella
- Form of spore (if present), which is ellipsoidal and not extending the mother cell
- Colour of the culture: light cream (brownish) to cream
- Morphology of colony (shape, elevations, margins) – varies with media and conditions of incubation)

Further information on parameters, distinguishing this strain of *B. subtilis* from others, is given in the ATCC report 1997 (BMF 2000-56) and by Gingras (1998, BMF 2000-65).

The original isolate of strain QST 713 of *B. subtilis* has been identified by additional biochemical and physiological key criteria, including (ATCC report, 1997, BMF 2000-56):
- Anaerobic growth
- pH of Voges-Proskauer reaction
- Maximum temperature growth
- Growth in 7 % NaCl
- Ability to form acid from glucose
- Ability to decompose casein
- Utilisation of citrate

Descriptions of corresponding standard methods of identification are given by Collins et al. (1989) and in a summarised form by Priest et al. (1988).
Slepecky (1992) reviews identification of and differences among *Bacillus* species.

The following data concerning methods are derived from Bellet (1998, BMF 2000-66) unless otherwise stated.

Methods for the identification of the micro-organism

During the fermentation process, at each seed transfer point, the identity of the fermented micro-organism is checked by streak plating and evaluation of the developing.

Confidential information about the details, see Annex C.

Methods for establishing purity of seed stock

Confidential information about the details, see Annex C.

Methods for providing information on possible variability of seed stock/ active organism

The use of pure seed stock for inoculation of production seed vials excludes the problem of variable content of active organism.
Purity is achieved by continuously controlling seed vials for quality according to microscopic as well as macroscopic morphology of colonies and by regular quality checks determining content of active ingredient in the broth or Technical Powder (by counts of colony forming units).

Methods to differentiate a mutant micro-organism from the parent wild strain

Each fermentation run is started with initial seed stock culture, which is maintained frozen in vials. Thus mutations in the original parent strain QST 713 are precluded.

Quality control measures applied to QST 713 Technical

Quality control comprises:

a) Determination of moisture content or dry weight
b) Determination of content of active ingredient
c) Tests for microbial contaminants
d) Detection of human pathogens

a) Determination of moisture content
Using an oven or infrared moisture analyser samples are analysed and the average reported. Details are given by Bellet (1998, BMF 2000-66).

b) Determination of content of active ingredient
Quantitative determination of viable *B. subtilis* is based on counts of colony forming units developing on agar after streak plating of QST 713 samples from serial dilution and incubating for 1 to 5 days. Details are given by Bellet (1998, BMF 2000-66). Further details on determining aerobic colony forming units (cfu) are given by Heins (1999).

A cfu count on the Technical Powder is not required when formulation to the end use product is consecutive to the fermenter run, but will be performed on the end use product instead.

c-d) Test for microbial contaminants and detection of human pathogens

Each fermentation run is checked for occurrence of contaminants and human pathogens in the broth. Additionally five batches have been produced for registration and toxicology testing of which two were analysed as Technical Powder diluted in water.
Test methods for human pathogens are performed by procedures that are either
- modifications of FDA Bacteriological Analytical Manual (BAM) or
- with procedures accepted by the FDA BAM methods, Association of Official Analytical Chemists (AOAC) Official Methods of Analysis or
- by accepted rapid test methods for identification.

Each contaminant will be identified in a tiered fashion to verify that it is not a human pathogen. Instructions for performance of these tests are reported in the Appendix of the above cited relevant Document (Bellet, 1998, BMF 2000-66). Considering the precautions and ingredients involved in fermentation there is no risk of relevant impurities of toxic or environmental concern to be formed or present in the fermentation material or end product.

Information about details, see Annex C.

Methods to determine storage stability of the micro-organism

Storage stability of QST 713 Technical has been determined covering a one-year period (Gingras, 1999). The employed test determined the microbiological titer (cfu/g) of five different lots of test substance at different time points up to one year following storage under warehouse conditions. Storage stability is indicated by consistent titer values.

B.5.1.2 Methods for the analysis of the preparation (Annex IIIB 5.1)

In principle *B. subtilis* is identified and analysed using biological methods, i.e. plating on organic growth media. Additionally the immediate microscopic appearance of a drop of liquid culture provides identification and is continually checked during the fermentation process. The growth of *B. subtilis* is continually monitored during the fermentation process both microscopically and by optical density.

The following data concerning methods are predominantly derived from Cunningham Hilbig (1999), unless otherwise stated.

B.5.1.2.1 Quality control measures applied to the production of QST 713 WP

Serenade™ WP consists of the strain QST 713 of dried *B. subtilis*, residual fermentation media and inert ingredients. A uniform product is achieved by employing standard fermentation and formulation processes, with each step being carefully performed, and by applying quality control.
Quality control (QC) samples are taken both at the end of the fermentation phase, from the broth, and at the end of the formulation phase, from the WP.

Quality control analysis includes:

- Determination of physical properties (moisture content and bulk density of WP and broth dry weight)
- Content of active ingredient by plate counts of colony forming units (cfu), for broth and WP.
- Determination of human pathogens and other contaminants is performed on the broth of each fermentation run (batch).
- Efficacy tests are performed on a subset of annually produced WP lots.
  Information about the details, see Annex C.

Each contaminant will be identified in a tiered fashion to verify that it is not a human pathogen. Details on methods are given for the Technical Product (Bellet, 1998, BMF 2000-66).

The fermentation process is the only phase that can support growth of contaminants and human pathogens, consecutive phases are not susceptible due to unfavourable conditions set by the parameters of post-fermentation process and by addition of ingredients with anti-microbial activity. Therefore, typically the broth is tested for contaminants.

B.5.2 Analytical methods to determine and quantify viable and non-viable residues of active organism, secondary metabolites and degradation products (Annex IIB 4.2; Annex IIIB 5.2)

Basically, determination of the active agent implies its isolation from media such as soil or water. Heat treatment of the water-suspended sample is the first step employed in order to destroy all vegetative cells but leave the heat resistant Bacilli endospores. Subsequent plating on enrichment media and selecting for colonies that exhibit certain morphological, optical and physiological characteristics comprise the species specific isolation and identification technique, as reported in chapter B.5.1 in this section.
The applied key characteristics used to identify strain QST 713 as *B. subtilis* are defined in the ATCC report (1997, BMF 2000-56).
Persistence studies on the survival of *B. subtilis* introduced into soils usually are performed with genetically marked strains that can be either spontaneous mutants or genetically engineered. Strains harbouring (plasmid bound) genes coding for specific antibiotic resistance (Asaka et al., 1996; Elsas et al., 1986) can be recovered from soil or other habitats by plating on agar amended with the respective antibiotic.

Information about details, see Annex C.

B.5.3 Evaluation and assessment

B.5.3.1 Formulation analysis

Adequate methodology exists for identification of *Bacillus subtilis*, strain QST 713, in the fermentation broth, the technical product, the plant protection product, environmental media (soil, water) and animal tissue. Techniques used to ensure a uniform product and assay methods for its standardization as well as information on quality control measures during production process were provided.

B.5.3.2 Residue analysis

The ingredients of Serenade™ WP are inert, non-toxic and pose no environmental or health risk. Thus, it can be concluded that information about behaviour of the active ingredient, *B. subtilis*, also applies to the formulated product.
Taking into account that the active substance, *B. subtilis*, is a non-pathogenic, non-hazardous micro-organism of ubiquitous distribution, and regarding the non-reactive, non-toxic properties of all ingredients comprising the product, no residues relevant to the safety of consumers, workers or the environment do occur. Therefore, methods for residue analysis are not required.

B.5.4 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[5]
AIIB-1.3; AIIB-2.5; AIIB-4.1	Anonymous	1997	Project Report SC 3817 - Bacterial Characterization ATCC. not GLP, unpublished BMF2000-56	Y	QST
AIIB-4.1	Collins, C.H., Lyne, P.M. and Grange, J.M.	1989	MICROBIOLOGICAL METHODS. not GLP, published Buttenworths, London (4095), Chapter 7: Identification Methods, 1989, 97-114 BMF2000-187	N	-
AIIB-4.1	Gingras, B.A.	1999	STORAGE STABILITY OF TECHNICAL QST 713 (FINAL REPORT). L08726 SN2 GLP, unpublished BMF2000-186	Y	QST

[5] Only notifier listed

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[5]
AIIB-4.1	Heins, S.D.	1999	METHODS FOR DETERMINATION OF AEROBIC COLONY FORMING UNITS UTILIZING SPIRAL PLATER (DSOP#: FM4201). GLP, unpublished BMF2000-185	Y	QST
AIIB-4.1	Priest, F.G. et al.	1988	A NUMERICAL CLASSIFICATION OF THE GENUS BACILLUS. not GLP, published J. Gen. Microbiol., Vol 134, 1988, 1847-1882 BMF2000-188	N	-
AIIB-1.3; AIIB-2.4; AIIB-4.1	Slepecky, R. A.	1992	What is a Bacillus?. not GLP, published in: Biology of Bacilli (Chapter 1) Doi, R. H. and Mc Gloughlin, M. (eds.) Application to Industry Buttenworth-Heinemann, Boston, 1992, 1-21 BMF2000-58	N	-
AIIB-2.1; AIIB-2.2; AIIB-2.4; AIIB-4.2	Asaka, O., Ano, T. and Shoda, M.	1996	Persistence of Bacillus subtilis RB14 and its derivative strains in soil with respect to the LPA-14 gene. not GLP, published J. of Fermentation and Bioengineering, Vol. 81, 1996, 1-6 BMF2000-92	N	-
AIIB-2.2; AIIB-2.5; AIIB-4.2	Campbell, R.	1989	Biocontrol on leaf surfaces. not GLP, published in: Biological control of microbial plant patho-gens Cambridge University Press, Cambridge, Department of Botany, University of Bristol Chapter 3, 1989, 66-94 BMF2000-100	N	-

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[5]
AIIB-2.4; AIIB-4.2; AIIB-7.1	Elsasv. J.D., Dijkstra A.F., Govaert J.M. and Veen v. J.A.	1986	Survival of Pseudomonas fluorescens and Bacillus subtilis introduced into two soils of different texture in field microplots. not GLP, published Federation of European Microbiological Societies, FEMS Microbiology Ecology 38, 1986, 151-160 BMF2000-146	N	-
AIIB-1.3; AIIB-2.1; AIIB-2.3; AIIB-2.4; AIIB-2.5; AIIB-2.6; AIIB-4.2	Priest, F. G.	1993	Systematics and Ecology of Bacillus. not GLP, published in: Bacillus subtilis and other gram-positive bacteria American Society of Microbiology, Washington D.C. (ed.), 1993, 3-16 BMF2000-57	N	-
AIIIB-2.2; AIIIB-4.4; AIIIB-5.1	Gingras, B. A.	1999	Storage Stability of Qst 713 Strain of Dried Bacillus subtilis with-Residual Fermentation media Identified as Qst 713 WP. Project No. L08726 SN9 GLP, unpublished PHY2000-721	Y	QST

Codes of owner

QST: AgraQuest, Inc.

Annex B

Bacillus subtilis strain QST 713

B-6: Toxicity, pathogenicity and infectivity

B.6 Toxicity, pathogenicity and infectivity

In the submitted study reports the calculation and evaluation of test results comply with U.S. provisions which in parts differ from the relevant European directive and lead to diverging classifications. The classification was performed according to EC-Commission directive of September 1st, 1993, on the approximation of the laws, regulations and administrative provision relating to the classification, packaging and labelling of dangerous substances (67/548/EEC).

Pending a final international agreement on data requirements for health evaluation of micro-organisms the present toxicological assessment of *Bacillus subtilis* considers the most recent recommendations as specified in annex IIB (Doc. 4992/I/95 - Draft 29/3 1999) and annex IIIB (Doc. 4993/I/95 - Draft 29/3 1999) of guideline 91/414 EEC. However, the submitted reports on the toxicity, pathogenicity, and infectivity studies and hence the edition of these studies by the Rapporteur are mainly based on the scheme proposed in earlier drafts of these annexes (revised 17 July 1997).

B.6.1 Step I - Basic studies (micro-organism) (Annex IIB 5.1)

B.6.1.1 Acute toxicity, pathogenicity and infectivity (Annex IIB 5.1.1)

Acute toxicity tests with QST 713 Technical, containing *Bacillus subtilis* strain QST 713 with residual fermentation media, demonstrate that this test substance is of a low order of acute toxicity to Sprague-Dawley rats (CD® rat strain) by the oral and respiratory routes, supporting LD_{50} values of greater than 5×10^8 cfu/kg bw following oral and intratracheal administration. QST 713 Technical is non-irritating to the skin and eye of the New Zealand White rabbit. A summary of the results from the acute toxicity studies with QST 713 Technical is presented in Table B.6.1-1.

Table B.6.1-1: Summary of the Acute Toxicity Studies/Step I for QST 713 Technical

Study Type	Strain	Results	Reference
LD_{50}, oral	CD® rats	NOEL >5×10^8 cfu/kg bw (males and females)[a]	Harrington (1998a)
LD_{50}, intratracheal	CD® rats	LD_{50} >5×10^8 cfu/kg bw (males and females)[b]	Harrington (1998b)
Rabbit Dermal Irritation	New Zealand White	Non-irritating	Bellet (1998b)
Rabbit Eye Irritation	New Zealand White	Non-irritating	Bellet (1998c)

[a]No treatment-related mortality or clinical signs of toxicity at the dose indicated.
[b]No treatment-related mortality at the dose indicated. No NOEL determined.

B.6.1.1.1 Acute oral toxicity, pathogenicity and infectivity (Annex IIB 5.1.1.1)

Report: Harrington, K. A. (1998a): Toxicity / pathogenicity testing of QST 713 following acute oral challenge in rats; IIT Research Institute Chicago, Illinois U.S.A.; unpublished; Laboratory Project ID L08726 SN4; dates of experimental work: Apr. 13, 1998 – May 6, 1998

Test Material: QST 713 Technical (dried *Bacillus subtilis* with residual fermentation media); Lot No. 8AQ07C2; Titer: 4,3 x 10^{10} cfu/g; no bacterial or fungal contamination; homogeneity Test (10 ml): positive.

Test Animals: CD®rats

GLP: Yes (self certification by the laboratory)

Test Method: EPA-Pesticide Assessment Guidelines, Subdivision M – Section Series 152A-10 (Microbial Pesticide Test Guidelines OPPTS 885.3050). Corresponds generally to EEC B1 - Directive 92/69/EEC (limit-test), and to OECD guideline 401

Deviations: Dose levels are not applicable to microbial preparations.

Acceptability: The study is considered to be acceptable.

Material and Methods:
The test substance was suspended in sterile water and administered to groups of male and female CD®rats (3 rats per group/sacrifice day/ sex) at a dose level of 1.13 x 10^8 cfu/ test animal (1 ml orally; the dose was consistent with EPA Subdivision M guidelines, Section Series 152A-10). The rats treated with technical grade test substance and the shelf control rats were housed together in the same room. This room was separate from a second room containing two additional control groups, i.e., the naive control group without any contact with the test substance and a group of rats treated with the killed test substance (by autoclaving at 121°C for 15 min). Rats were sacrificed at Day 0, 3, 7, and 14. Observed parameters consisted of clinical and necropsy observations, body weights, body weight gains, organ weights and microbial enumeration. Clearance of *Bacillus subtilis* was determined in selected rat tissues (blood, brain, lungs, spleen, liver, kidneys, mesenteric lymph nodes, stomach, small intestines, caecum and feces) by plating analysis, for the treatment group both without and with heat treatment of the tissue samples (at 65°C for 30 min) to inactivate vegetative or heat-intolerant spores.

Findings:
The results indicate no evidence of toxicity or pathogenicity related to treatment with QST 713 Technical at the oral dose administered. No deaths occurred and no adverse clinical observation were noted. No treatment-related effects in body weight or body weight gain were observed. There were no treatment-related differences in relative organ/tissue weights.

No test substance (viable *Bacillus subtilis*) was found neither in the naive control and shelf control goups nor in the animals receiving the killed test substance (Tables B.6.1-2 and B.6.1-3). In the test substance group, *Bacillus subtilis* was detected, pre- and post-heat treatment, in stomach and intestines, caecum and feces of male and female rats, plus in the lungs, liver and mesenteric lymph nodes of female rats at the day of administration only (Table B.6.1-3). Highest numbers of cfu were found in the stomach. The numbers of cfu increased in the feces of test substance-treated rats until Day 3 in males and females. Within 14 days (after dosing) test substance was cleared from all tissues tested. Necropsy findings were normal for all animals.

Table B.6.1-2: Recovery of *Bacillus subtilis* (cfu) from tissues of male rats on Day 0, 3, 7, and 14 after oral administration

Tissue/ body fluid	Naive control	Shelf control	Killed test substance	Test substance[1]			
	Day 0 - 14	Day 0 - 14	Day 0 - 14	Day 0	Day 3	Day 7	Day 14
Blood	-[2]	-	-	-	-	-	-
Lungs	-	-	-	-	-	-	-
Spleen	-	-	-	-	-	-	-
Liver	-	-	-	-	-	-	-
Kidneys	-	-	-	-	-	-	-
Brain	-	-	-	-	-	-	-
Mesenteric lymph nodes	-	-	-	-	-	-	-
Stomach & Small intestines	-	-	-	8.2×10^7	8.5×10^0	-	-
Caecum	-	-	-	9.7×10^3	2.3×10^2	1.0×10^1	-
Feces	-	-	-	1.2×10^3	2.5×10^3	2.7×10^2	-

[1]Determination of titer pre-heat treatment of tissues (geometric mean of cfu/ tissue or ml blood)
[2] - = below detection limit (< 30 cfu/tissue or ml blood)

Table B.6.1-3: Recovery of *Bacillus subtilis* (cfu) from tissues of female rats on Day 0, 3, 7, and 14 after oral administration

Tissue/ body fluid	Naive control	Shelf control	Killed test substance	Test substance[1]			
	Day 0 - 14	Day 0 - 14	Day 0 - 14	Day 0	Day 3	Day 7	Day 14
Blood	-[2]	-	-	-	-	-	-
Lungs	-	-	-	3.2×10^1	-	-	-
Spleen	-	-	-	-	-	-	-
Liver	-	-	-	3.9×10^1	-	-	-
Kidneys	-	-	-	-	-	-	-
Brain	-	-	-	-	-	-	-
Mesenteric lymph nodes	-	-	-	7.5×10^2	-	-	-
Stomach & Small intestines	-	-	-	1.1×10^8	1.4×10^1	-	-
Caecum	-	-	-	1.2×10^4	4.5×10^1	-	-
Feces	-	-	-	3.2×10^3	3.4×10^3	1.2×10^3	-

[1]Determination of titer pre-heat treatment of tissues (geometric mean of cfu/ tissue or ml blood)
[2] - = below detection limit (< 30 cfu/tissue or ml blood)

Discussion:
Within the study design parameters measured, QST 713 Technical was not found to be toxic or pathogenic at the dose administered by the oral route to rats. Detection of test substance (pre- and post-heat treatment) from the stomach and small intestines, caecum and feces of treated rats on Day 0 was consistent with oral administration. By Day 14, test substance was cleared from all tissues tested.

Conclusion:
The oral LD_{50} as well as the lowest dosage causing first symptoms (LOEL) are well above the limit dose of 1.13×10^8 cfu/ test animal; corresponding to approximately 5×10^8 cfu/ kg bw. Therefore, on the basis of the mortality results and the absence of any clinical signs the active substance, QST 713 Technical, can be classified as non-toxic by the oral route (no labelling requirements according to EC directive 67/548/EEC).

B.6.1.1.2 Acute intratracheal toxicity, pathogenicity and infectivity (Annex IIB 5.1.1.2)

Report: Harrington, K. A. (1998b): Toxicity/ pathogenicity testing of QST 713 following acute intratracheal challenge in rats; IIT Research Institute Chicago, Illinois U.S.A.; unpublished; Laboratory Project L08726 SN6; dates of experimental work: Apr. 13, 1998 – May 29, 1998

Test Material: QST 713 Technical (dried *Bacillus subtilis* with residual fermentation media; Lot No. 8AQ07C2); Titer: 4.3×10^{10} cfu/g; no bacterial or fungal contamination; homogeneity Test (10 ml): positive.

Test Animals: CD®rats

GLP: Yes (self certification by the laboratory)

Test Method: EPA-Pesticide Assessment Guidelines, Subdivision M – Section Series 152A-12 (Microbial Pesticide Test Guidelines OPPTS 885.3150).

Deviations: No OECD guideline applicable.

Acceptability: The study is considered to be acceptable.

Material and Methods:
The test substance was suspended in sterile water and intratracheally administered to groups of male and female CD®rats (5 rats per group/sacrifice day/ sex) at a dose level of approximately 1.2×10^8 cfu/ test animal (0.1 ml); the dose was consistent with EPA Subdivision M guidelines, Section Series 152A-12. Control groups (as in B.6.1.1.1): naive control, shelf control (housed with the treated group) and a control group receiving the heat-killed test substance (rendered non-viable by autoclaving at 121°C for 15 minutes). Rats were sacrificed at day 0, 7, 21 and 35. Observed or measured parameters consisted of clinical and necropsy observations, body weights, body weight gains, organ weights and microbial enumeration. Clearance of *Bacillus subtilis* in rat tissues was determined by plating analysis, for the treatment group both without and with heat treatment of the tissue samples (to inactivate vegetative/heat intolerant spores).

Findings:
No deaths occurred, and except for one male rat (of 40) showing rough hair coat on Day 0 following dosing, no adverse clinical signs were observed for any other animal during the study. Sole necropsy findings were: mottled lung parenchyma in animals examined on Day 0 following test substance administration. Body weight gain significantly decreased during the first week after administration of test substance. During the 4th week male rats compensated this decrease while female rats had significantly decreased body weight gains on Day 21. Increased relatively lung weights on Day 0 and Day 7 (males only) and decreased relative liver weights (males) on Day 7 were found compared to the naive control group. Increased relative lung weights and decreased relative spleen weights were found in treated female rats on Day 0. There were no other treatment-related differences in relative organ/tissue weights.

No test substance was detected in any tissue of the control groups, including the group that received the heat-killed test substance. Decreasing titer values of test substance were detected in the lungs and associated lymph nodes of treated rats, up to Day 35 (end of observation period), both pre- and post-heat-treatment of tissues (Tables B.6.1-4 and B.6.1-5). Post-heat-treatment of tissues, test substance was also detected in liver, kidney and spleen (up to Day 7). By Day 21 test substance numbers were significantly decreased or below detection limit from all tissues tested. There was no evidence of germination or vegetative growth of *B. subtilis* in the rats.

Table B.6.1-4: Recovery of *Bacillus subtilis* (cfu) from tissues of treated male rats on Day 0, 7, 21, and 35 after intratracheal administration

Tissue/ body fluid	Test substance[1]			
	Day 0	Day 7	Day 21	Day 35
Blood	-	-	-	-
Lungs & Lymph nodes	6.3×10^7	4.5×10^7	1.6×10^6	8.7×10^4
Spleen	2.3×10^0	3.3×10^1	-	-
Liver	7.9×10^2	1.4×10^1	-	-
Kidneys	1.2×10^1	-	-	-
Brain	-	-	-	-
Caecum	-	-	-	-

[1]Determination of titer post-heat treatment of tissues (geometric mean of cfu/ tissue or ml blood)
[2] - = below detection limit (< 30 cfu/tissue or ml blood)

Table B.6.1-5: Recovery of *Bacillus subtilis* (cfu) from tissues of treated female rats on Day 0, 7, 21, and 35 after intratracheal administration

Tissue/ body fluid	Test substance[1]			
	Day 0	Day 7	Day 21	Day 35
Blood	-	-	-	-
Lungs & Lymph nodes	7.1×10^7	3.2×10^7	2.5×10^6	6.6×10^4
Spleen	-	1.3×10^2	-	-
Liver	1.2×10^2	1.1×10^1	-	-
Kidneys	1.1×10^1	-	-	-
Brain	-	-	-	-
Caecum	-	-	-	-

[1]Determination of titer post-heat treatment of tissues (geometric mean of cfu/ tissue or ml blood)
[2] - = below detection limit (< 30 cfu/tissue or ml blood)

Discussion:
Within the study design parameters measured, QST 713 Technical was found not to be pathogenic in CD rats following intratracheal administration. The treatment-related reductions of body weight gain were only transient. Increased lung weight in treated rats may be due to the physical presence of the test substance along with the host defence cellular infiltrates and associated edema. Detection of test substance from the lungs and associated lymph nodes of treated rats on Day 0, and transiently from the spleen, liver and kidneys, is consistent with intratracheal administration of *Bacillus subtilis*. Complete clearance from all tissues was estimated to occur within approximately 108 days from challenge. Clearance of test substance was consistent with normal clearance observed with *Bacillus* spores; extended clearance time is often observed following challenge with *Bacillus* spores and there was no evidence of germination or vegetative growth of the test substance in rats. Necropsy findings consisted of mottled lung parenchyma for all Day 0 test substance-treated animals. No other test substance-related necropsy observations were noted.

Conclusion:
The LD_{50} following intratracheal administration of QST 713 Technical is well above the limit dose of 1.2×10^8 cfu/ test animal, corresponding to approximately 5×10^8 cfu/ kg bw. The generally minor and short-termed clinical signs and necropsy findings show that intratracheally applied *Bacillus subtilis* can be evaluated as a low health risk.

B.6.1.1.3 Intraperitoneal/Subcutaneous single dose (Annex IIB 5.1.1.3)

This test was not performed, but the conducted intravenous administration study in rats (see B.6.3.1.1) is considered an appropriate compensation, since intravenous exposure represents more severe test conditions.

B.6.1.1.4 Skin irritation (Annex IIB 5.1.1.4)

Report: Bellet, E. (1998b): Primary dermal irritation in rabbits with QST 713 TP; Chrysalis, Olyphant, PA 18447, U.S.A.; unpublished; Study Number: 0420XA54.004; dates of experimental work: June 25– June 28, 1998.

Test Material: QST 713 Technical (dried *Bacillus subtilis* with residual fermentation media; Lot No.: 8AQ07C2). Titer of this lot (determined in Harrington 1998a) is ~ 4.3 x 10^{10} cfu/g

Test Animals: New Zealand White rabbits

GLP: Yes (self certification by the laboratory)

Test Method: EPA-Pesticide Assessment Guidelines, Subdivision F, (No. 81-5) Corresponds to EEC B4 – Directive 92/69/EEC, and to OECD guideline 404 (applying to chemical substances)

Deviations: No information on homogeneity or stability of the test compound is given in the study report.

Acceptability: The study is considered to be acceptable.

Material and Methods:
500 mg of moistened (~ 2.1 x 10^{10} cfu, as dose level/animal) test substance was applied to one intact site on the clipped dorsal trunk of 3 male and 3 female New Zealand White rabbits and held in place with a semi-occlusive wrapping. To help facilitate absorption, the test article was moistened with 0.3 ml saline before covering the treated area with a gauze patch. At the end of a 4-hour exposure period, sites were unwrapped and residual test article was removed using water. Observations for dermal irritation were recorded at approximately 30-60 minutes and at 24, 48 and 72 hours after unwrapping. Grading of irritation is according to the method of Draize. Body weights were recorded prior to treatment initiation and at the end of the observation period.

Findings:
No mortality was observed. Slight erythema symptoms were observed at 30 min and at 24 h following application, symptoms cleared by 48 h. No other dermal signs were noted. The primary irritation index was calculated to be 0.3 (on a scale 0 to 4). No significant effects on body weights were noticed.

Conclusion:
QST 713 TP caused very slight irritation symptoms after 4 h of dermal exposure. According to EC directive 67/548/EEC QST 713 TP is classified as **non-irritant**.

B.6.1.1.5 Eye irritation (Annex IIB 5.1.1.5)

Report: Bellet, E. (1998c): Primary eye irritation in rabbits with QST 713 TP; Chrysalis, Olyphant, PA 18447, U.S.A.; unpublished; Study Number: 0421XA54.004, dates of experimental work: July 10 – July 14, 1998.

Test Material: QST 713 Technical (dried *Bacillus subtilis* with residual fermentation media; Lot No.: 8AQ07C2). Titer of this lot (determined in Harrington 1998a) is ~ 4.3 x 10^{10} cfu/g, conclusively 81 to 100 mg contain ~ 3.5 – 4.3 x 10^{9} cfu, as dose level/animal.

Test Animals: New Zealand White rabbits

GLP: Yes (self certification by the laboratory)

Test Method: Dose selection according to EPA Pesticide Assessment Guidelines, subdivision F No. 81-4 (1984), partly also applying to scale for scoring ocular lesions.
Tabulating of test article according to Addendum 2 on EPA Pesticide Assessment Guidelines – Eye Irritation (1988)
Corresponds to EEC B5 - Directive 92/69/EEC, and to OECD guideline 405 (applying to chemical substances)

Deviations: No information on homogeneity or stability of the test compound is given in the study report.

Acceptability: The study is considered to be acceptable.

Material and Methods:
Packed into a 1cc syringe to a 0.1 ml volume (sample weight 81-99.9 mg) the test substance was instilled into the conjunctivae sac of the right rabbit eye (3 males, 3 females), with the left eye (untreated) serving as a control. Reactions were recorded: initially, at approximately 1, 24, 48 and 72 hours and on Day 4 following administration. Grading of irritation is according to the method of Draize.

Findings:
Main affected area was the conjunctivae predominantly showing redness in all the animals; the redness persisted through the 72-hour observation period in half of the animals. Slight swelling (chemosis) mainly occurred within the first hour following application. The iris exhibited only lowest grades considered positive. All symptoms had ceased within the 4-day observation period.

The reported calculation and evaluation of the Draize scores is based upon the U.S. provisions which differ fundamentally from the relevant EC directive 67/548/EEC. The adopted calculation gives following values, referring to each symptom separately:

Symptom	Mean score value	Classification
Cornea opacity	0	none
Iris lesions	<1	none
Redness of conjunctivae	<1	none
Chemosis of conjunctivae	<1	none

Conclusion:
QST 713 Technical was determined to be **non-irritant** according to the relevant EC directive 67/548/EEC.

B.6.1.1.6 Skin sensitisation (Annex IIB 5.1.1.6)

A skin sensitisation test has been performed with the wettable powder formulation Serenade™ WP (see B.6.7.3). Given the sensitising potential of the formulation and the absence of any data on skin sensitization by the active ingredient, QST 713 Technical is suggested to be classified as a skin sensitiser.
It should be emphasised that "The available methods for testing dermal sensitisation are not suitable for testing micro-organisms." (Doc. 4992/I/95 - Draft 29/3 1999).

B.6.1.2 Genotoxicity testing (Annex IIB 5.1.2)

The genotoxic potential of this micro-organism has not been examined in specific studies since there is no evidence coming from the literature that any genotoxic substance was produced. *Bacillus subtilis* has never been associated with cancerogenesis as the causative agent or merely with entry into mammalian cells. Taking into account its ubiquitous distribution, even in foods, genotoxicity testing appears to be dispensable.

B.6.1.3 Cell culture studies (Annex IIB 5.1.3)

No cell culture studies were performed since *Bacillus subtilis*, as a natural soil inhabitant, does not enter the cytoplasm to replicate intracellularly. The members of the species *Bacillus subtilis* do not show specific attachment mechanisms typically found in organisms capable of colonizing humans (EPA 1997).

B.6.1.4 Short-term toxicity, pathogenicity and infectivity (Annex IIB 5.1.4)

No such study was performed in consistence with the results of the above cited acute toxicity studies proving the absence or minor significance of clinical signs.
An overall low health risk imposed by *Bacillus subtilis* may also be derived from the safe application of *B. subtilis* in the industrial setting, e.g. large scale enzyme production, and regarding its use in vaccine production (see TAIRA et al 1989).
A potential escape of the host defence mechanisms after repeated doses may be excluded based on the results of an intravenous challenge with 35-day observation period, which caused no adverse impacts (see B.6.3.1.1).

On the other hand, *Bacillus subtilis* has been isolated in some cases of food poisoning and from human infections in very few patients with a compromised immune status (B.6.4.3). Given the production of toxicologically relevant substances (e.g., subtilisin) and the slow clearance of *Bacillus subtilis* spores from some rat tissues after intratracheal and intravenous administration, a repeated dose inhalation study should be required in this special case.

B.6.1.5 Pathogenicity and infectivity under immunosuppression (Annex IIB 5.1.5)

Bacillus subtilis can grow at temperatures higher than 32°C, as given in human bodies, but it is known as usually non-pathogenic. Therefore no epidemiological studies were performed and no medical surveillance programme was conducted. Following statements can be inferred from literature:

- *Bacillus subtilis* has a low virulence and low risk potential for human health and is regarded as non-pathogenic and non-toxic (EPA 1997; de BOER & DIDERICHSEN 1991).
- *Bacillus subtilis* does have a potential in eliciting allergic reactions in individuals repeatedly exposed to the secreted proteinaceous compound *subtilisin* (EPA 1997).
- Publications on clinical cases suggest no invasive properties of *Bacillus subtilis*: in some cases *Bacillus subtilis* was isolated from surgical wound or tumor drainages, but it remained locally restricted and did not influence the course of wound healing; only highly immuno-suppressed patients were reported to have suffered from dissipating bacterial infections caused by *Bacillus subtilis* (and other species) (IHDE & ARMSTRONG 1973; BOER & DIDERICHSEN 1991; B.6.4.3).

These findings suggest that under normal health conditions no pathogenicity and infectivity of *Bacillus subtilis* is expected to occur, especially in view of the given ambient exposure towards this ubiquitous bacteria. It can be assumed that the number of micro-organisms challenging an individual must be very large and the immune status of this individual very poor to facilitate an infection with *Bacillus subtilis*. The former situation might be given for a limited number of workers and operators wich can be assumed to be in a sufficient general health state whereas the latter one would apply to some persons among the general population who are usually not expected to be exposed to a high number of bacteria of this species.

B.6.2 Step II - Additional studies (micro-organism) (Annex IIB 5.2)

Toxicity studies conducted under Step I did not show any significant health effects.
In addition to studies referred to under Step I, an acute dermal toxicity test was conducted as basically required by the U.S. EPA. The test results are reported below.

B.6.2.1 Acute percutaneous toxicity, pathogenicity and infectivity (Annex IIB 5.2.1)

Report: Findlay, J. (1998): Acute dermal toxicity/ pathology study of QST 713 in rabbits; IIT Research Institute, Chicago, Illinois, U.S.A.; Laboratory Project ID L08726 SN7; unpublished; dates of experimental work: Apr. 1 – April 29, 1998.

Test Material: QST 713 Technical (dried *Bacillus subtilis* with residual fermentation media; Lot No.: 8AQ07C2; reported titer: 4.7×10^{10}cfu/g

Test Animals: New Zealand White rabbits

GLP: Yes (self certification by the laboratory)

Test Method: EPA - Pesticide Assessment Guidelines, Subdivision M – Section Series 152A-10 (Microbial Pesticide Test Guidelines OPPTS 885.3100)
Corresponds generally to EEC B3 - Directive 92/69/EEC (limit-test) and to OECD guideline 402 applying to chemical substances

Deviations: No information on homogeneity or stability of the test compound is given in the study report.

Acceptability: The study is considered to be acceptable.

Material and Methods:
Individual doses of 2 g/kg body weight, suspended in 3 ml sterile water, were administered in paste form to the shaved backs of five male and five female New Zealand White rabbits and the test site was covered with a 12.8 x 11.5 cm surgical dressing. The wrappings were removed after 24 hours and the application sites were wiped clean of excess test substance. Based on the animal body weights, individual test substance doses ranged between 2.3 – 2.73 $\times 10^{11}$ cfu. Daily observations were recorded for 14 days.

Findings:
No deaths and no systemic toxicity signs occurred during the study. All rabbits gained weight during the study. All animals exhibited signs of dermal irritation consisting of erythema, edema, eschar formation and necrosis during the study. In addition, four animals exhibited cracking of the skin. Slight to moderate edema, severe erythema and eschar formation were observed in all rabbits and multiple sores were observed in nine rabbits within hours following unwrapping on Day 2. Eschar formation persisted until Days 5-13 in all animals, with the exception of one animal that exhibited eschar formation at the study termination (Day 14). The severity of erythema and edema began to decrease on Day 6 and Day 8, respectively, and continued to do so until the end of the study (Day 14), at which time only one rabbit exhibited severe erythema and very slight edema. All but one animal exhibited superficial flaking of the skin beginning on Days 4-6 and all were devoid of it by Day 8. New/repaired skin was seen in all animals beginning on Days 5-7.

Discussion: No overt sign of systemic toxicity were observed in any rabbit during the study. All rabbits showed varying degrees of dermal irritation (erythema, edema, eschar formation, sores and necrosis). Superficial flaking of the skin appeared frequently, partly persisting at the study termination. All, but one female that continued to exhibit very slight edema and severe erythema/eschar formation, had completely recovered from signs of dermal irritation at the end of the observation period; new or repaired skin appeared at the application site.

Conclusion:
Following dermal administration of QST 713 Technical, the LD_{50} is well above the limit dose of 2 mg/kg bw (i.e., 2.3 – 2.73 x 10^{11} cfu/ test animal). The results show that the active substance, *Bacillus subtilis*, can be classified as non-toxic (no labelling requirements according to EC directive 67/548/EEC).

B.6.2.2 Genotoxicity – In-vivo studies in germ cells (Annex IIB 5.2.2)

Based on the information given in B.6.1.2 *Bacillus subtilis* is not expected to exert any genotoxic effect on germ cells.

B.6.3 Step III - Specific toxicity, pathogenicity and infectivity studies under immunosuppression (micro-organism) (Annex IIB 5.3)

Toxicity tests conducted under STEP I did not show any health effects. An additional acute percutaneous study also did not reveal any significant systemic toxicity on exposure to the test substance QST 713 TP. Strain QST 713 of *Bacillus subtilis* does not produce any toxins. Thus, any specific toxicity, pathogenicity or infectivity studies were not conducted. In addition to studies referred to under Step I and II, an acute intravenous toxicity test was conducted as basically required by the U.S. EPA. The test results are reported below.

B.6.3.1 Acute intravenous toxicity, pathogenicity and infectivity (Annex IIB 5.3.1)

Report:	Harrington, K.A. (1998c): Toxicity/ pathogenicity testing of QST 713 following acute intravenous challenge in rats; IIT Research Institute, Chicago, Illinois, U.S.A.; Laboratory Project ID L08726 SN5; unpublished; dates of experimental work: Apr. 13, 1998 – May 28, 1998.
Test Material:	QST 713 Technical (dried *Bacillus subtilis* with residual fermentation media; Lot No.: 8AQ07C2; reported titer: ~ 4.3 x 10^{10} cfu/g).
Test Animals:	CD®rats
GLP:	Yes (self certification by the laboratory)
Test Method:	EPA-Pesticide Assessment Guidelines, Subdivision M – Section Series 152A-13; Microbial Pesticide Test Guidelines OPPTS 885.3200; no bacterial or fungal contamination; homogeneity Test (10 ml): positive.

Deviations: No OECD guideline applicable

Acceptability: The study is considered to be acceptable.

Material and Methods:
Groups of male and female CD®rats (3 rats per group/sacrifice day/ sex) were dosed intravenously with ~ 9.4 x 10^6 cfu in a 0.5 ml volume (suspended in sterile water). This dose was consistent with EPA-Pesticide Assessment Guidelines, Subdivision M – Section Series 152A-13 (1 x 10^7 cfu/animal). Control groups (as in B.6.1.1.1): naive control, shelf control and a control group receiving the heat-killed test substance. Rats were sacrificed following dosing at Day 0, 7, 21 and 35.

Clearance of *Bacillus subtilis* in rat tissues was determined by plating analysis, for the treatment group both without and with heat treatment of the tissue samples (to inactivate vegetative/heat intolerant spores).

Findings:
Neither deaths nor adverse clinical signs or gross lesions at necropsy were observed during the study. No statistically significant treatment-related effects on body weight, body weight gain or relative organ/tissue weights were observed during the observation period of 35 days.

Test substance (viable *Bacillus subtilis*) was detected in the blood, liver, lungs, spleen and kidneys of treated male and female rats on Day 0 (pre- and post-heat treatment). Clearance of test substance occurred in most tissues within the observation period (by Day 35), reduced levels of test substance were found in spleen (pre- and post-heat treatment) and liver (post-heat treatment) of dosed rats (Tables B.6.3-1 and B.6.3-2). There was no evidence of germination or vegetative growth of QST 713 Technical in the rats. Complete clearance from all tissues was estimated to occur within approximately 80 days from challenge.

Table B.6.3-1: Recovery of *Bacillus subtilis* (cfu) from tissues of test substance-treated male rats on Day 0, 7, 21, and 35 after intravenous administration[1]

Tissue/ body fluid	Day 0	Day 7	Day 21	Day 35
Blood	4.4 x 10^2	3.5 x 10^1	-[2]	-
Lungs	5.4 x 10^5	3.7 x 10^3	7.1 x 10^1	-
Spleen	4.4 x 10^5	2.9 x 10^5	1.7 x 10^4	1.7 x 10^3
Liver	3 x 10^6	1.7 x 10^6	2.5 x 10^4	1.9 x 10^2
Kidneys	4.7 x 10^3	2.3 x 10^3	-	-
Brain	-	-	-	-
Mesenteric lymph nodes	-	-	-	-
Caecum	-	-	-	-

[1]determination of titer post-heat treatment of tissues (geometric mean of cfu/ tissue or ml blood))
[2] - = below detection limit (< 30 cfu/tissue or ml blood)

Table B.6.3-2: Recovery of *Bacillus subtilis* (cfu) from tissues of test substance-treated female rats on Day 0, 7, 21, and 35 after intravenous administration[1]

Tissue/ body fluid	Day 0	Day 7	Day 21	Day 35
Blood	3.2×10^2	1.6×10^2	-[2]	-
Lungs	4.4×10^5	3.2×10^3	5.1×10^1	-
Spleen	3.4×10^5	2.0×10^5	2.1×10^4	1.2×10^3
Liver	2.5×10^6	2.2×10^6	7.4×10^4	1.5×10^3
Kidneys	2.5×10^3	2.1×10^3	-	-
Brain	-	-	-	-
Mesenteric lymph nodes	-	-	-	-
Caecum	-	-	-	-

[1]determination of titer post-heat treatment of tissues (geometric mean of cfu/ tissue or ml blood))
[2] - = below detection limit (< 30 cfu/tissue or ml blood)

Discussion: QST 713 Technical caused no toxic or pathogenic effects when administered intravenously to rats. Detection of *Bacillus subtilis* from blood and organs was consistent with the intravenous route. A relatively slow clearance from liver and spleen was observed in male and female rats within 35 days. Clearance of test substance was consistent with normal clearance observed with *Bacillus* spores; extended clearance time is often observed following challenge with *Bacillus* spores and there was no evidence of germination or vegetative growth of the test substance in rats. Necropsy findings were normal for all animals.

Conclusion:
Following intravenous administration of QST 713 Technical, the LD_{50} as well as the lowest dosage causing first symptoms (NOEL) are well above the limit dose of 9.4×10^6 cfu/ test animal. The results show that the active substance, *Bacillus subtilis*, is non-toxic by the intravenous route.

B.6.4 Medical data (Annex IIB 5.4)

The EPA (1997) concluded that *Bacillus subtilis* is not a frank human pathogen, nor is it toxigenic like some other members of the genus. The virulence characteristics of the micro-organism are low, but on several occasions *Bacillus subtilis* has been isolated from human infections. Moreover, there have been several reported cases of food poisoning attributed to large numbers of *Bacillus subtilis* contaminated food. Thus, this micro-organism could be expected to temporarily inhabit the skin and gastrointestinal tract of humans, but it is doubtful that it would colonize other sites in the human body. *Bacillus subtilis* has been shown to be capable of producing lecithinase (an enzyme which disrupts membranes of mammalian cells), however, no correlation between lecithinase production and human disease has been found. Furthermore, *Bacillus subtilis* produces an extracellular toxin known as subtilisin which has very low toxigenic properties (GILL 1982) but is capable of causing allergic reactions in individuals who are repeatedly exposed to it (EPA 1997).

Different antibiotic molecules were identified as products from different strains of *Bacillus subtilis*. However, no antibiotics used in human or animal medicine are known to be produced.

Bacillus species, including *Bacillus subtilis*, were isolated from infected patients (mainly from their wound drainage) and found to be uniformly resistant to a variety of antibiotics (penicillin, ampicillin, oxacillin, methicillin and colistin (IHDE & ARMSTRONG 1973). However, no resistance genes against antibiotics which are used in animal or human medicine are inherent to strain QST 713 of *Bacillus subtilis*. Spontaneous mutations within the seed vials used for fermentation are excluded since the culture is consistently stored at –80°C.
Conclusively the application of Serenade™ WP does not contribute to or enhance the dispersal of antibiotic resistance in human pathogens.

B.6.4.1 Medical surveillance on manufacturing plant personnel (Annex IIB 5.4.1)

No medical observations on plant personnel were conducted. Exposure to *Bacillus subtilis* in the manufacturing plant will be minimal due to rigorous application of Good Manufacturing Practice (GMP), quality controls and due to protective equipment worn by the plant workers (Document Submission Template to U.S. BPPD – Biopesticides and Pollution Prevention Division, 1998). The EPA (1997) states that the only human health concern for workers in the fermentation facility is the potential of *Bacillus subtilis* to elicit allergic reactions in individuals repeatedly exposed to subtilisin. Sensitization of workers to subtilisin may be a problem in facilities where exposure to high concentration of this compound may occur. This risk is minimised by appropriate limits set by the U.S. OSHA (Occupational Safety and Health Administration) for subtilisin in the industrial setting.

B.6.4.2 Sensitisation/allergenicity observations (Annex IIB 5.4.2)

The potential for allergic reactions elicited by the proteinaceous compound subtilisin has been reported by the EPA (1997; see B.6.4.1). A case of familial hypersensitivity pneumonitis induced by *Bacillus subtilis* has been described by JOHNSON et al. (1980). Symptomatic members of the family demonstrated positive bronchoprovocation responses and lymphoproliferative responses to the vegetative cell extract of *Bacillus subtilis*; the latter response has also been found in one additional patient tested with the spore form of *Bacillus subtilis.*

B.6.4.3 Direct observation, e.g. clinical cases (Annex IIB 5.4.3)

Infections attributed to *Bacillus subtilis* include bacteremia, endocarditis, pneumomia, and septicemia. However, these infections were found in patients in compromised immune states. There must be immunosuppression of the host followed by inoculation in high numbers before infection with *Bacillus subtilis* can occur. Clinical cases have been investigated by IHDE and ARMSTRONG (1973) over a 6-year period. In twelve patients Bacillus species were determined to be present. They report that disseminated bacterial infections by *Bacillus subtilis* and other bacteria developed in two patients with acute leukemia who were under intense chemotherapy and finally died of their infections. *Bacillus subtilis* isolates from the remaining ten patients were locally restricted to surgical wound or tumor drainages and did not appear to affect wound healing. Other *pathogenic* bacteria were sometimes present in such culture material as well. The authors conclude that the presence of Bacillus species seems to indicate the infection of a wound or tumor mass. With the exception of the two immunocompromised patients no colonization of other organs or tissues took place.

From a review of additional and more recent references on clinical cases which partly were related to GRAS petitions published by the U.S. Food and Drug Administration, de BOER and DIDERICHSEN (1991) conclude that no case demonstrating invasive properties of *Bacillus subtilis* was described and that the 50 reported *Bacillus subtilis* infections (covering a 20-year period) were associated with drug abusers or severely debilitated patients. Under safety aspects they assume that due to the ubiquitous distribution of *Bacillus subtilis* it is inevitable that sometimes it may be found in association with other bacteria in infected humans.

Bacillus subtilis has been isolated in some cases of food poisoning, but the number of episodes is low. Thus, KARMER & GILBERT (1989) reported on only 49 episodes in the UK in the period 1975-1986. Exact and reliable figures are difficult to obtain, since *B. cereus* (a well-established cause of food poisoning) sometimes may have been classified as *Bacillus subtilis*. As a consequence, there are very few examples of *Bacillus subtilis* as the confirmed cause of food poisoning.

A case of *Bacillus subtilis* eye infection related to contamination of contact lenses has been reported by DONZIS et al. (1988).

B.6.4.4 Observations on exposure of the general population and epidemiological studies (Annex IIB 5.4.4)

The general population already is exposed to *Bacillus subtilis* since it is an ubiquitous micro-organism which inhabits primarily the soil environment and plant residues but has also been reported to occur in the immediate environment of humans, such as the kitchen (de BOER & DIDERICHSEN 1991). *Bacillus subtilis* is not pathogenic or toxic as proved by the submitted toxicological studies and e.g. has been shown to clear the body (of tested rats) after oral intake within 14 days (see B.6.1.1.1). Based on these findings no epidemiological studies have been performed, nor are corresponding reports available from the open literature.

The apparent lack of naturally induced interspecies gene transfer and consequently the improbability of a potential shifting of resistance genes into human pathogens supports the safety of *Bacillus subtilis.*

The commercial application of *Bacillus subtilis* in the Japanese food “Natto” and the prevalence of its endospores (e.g. in spices) should be considered in evaluating the safety of this micro-organism. The employment of *Bacillus subtilis* as hosts for vaccine or diagnostic antigen production in medical applications supports its low risk potential for humans (TAIRA et al 1989).

B.6.4.5 Proposed treatment: first aid, medical treatment (Annex IIB 5.4.5)

In cases of direct contact to *Bacillus subtilis* material occurs the following first behavioural steps are to be carried out according to the applicant:

- If inhaled: move to fresh air
- If in contact with eyes: flush eyes with plenty of water
- If in contact with skin, open cuts or wounds: wash skin with soap and water
- If swallowed: immediately give large amounts of water

Specific medical treatment following inhalative, oral or eye exposure is not required since *Bacillus subtilis* is not pathogenic or toxic. Following direct contact of *Bacillus subtilis* to open cuts or wounds preventively the relevant sites should be disinfected.

B.6.5 Summary of mammalian toxicity, pathogenicity and infectivity and overall evaluation (Annex IIB 5.5)

The acute toxicity and primary irritation studies with QST 713 Technical, containing *Bacillus subtilis* with residual fermentation media, are summarised in Table B.6.5-1.

Table B.6.5-1: Summary table of acute toxicity and primary irritation studies

Oral				
Species	Vehicle	Sex	NOEL (cfu/animal)	LD_{50} (cfu/animal)
Rat	Sterile water	3 per group/ sacrifice day/ sex	> 1.13 x 10^8	> 1.13 x 10^8
Intratracheal				
Species	Vehicle	Sex	NOEL	LD_{50} (cfu/animal)
Rat	Sterile water	5 per group/ sacrifice day/ sex	Not determined	> 1.2 x 10^8
Primary dermal irritation				
Species	Vehicle	Sex	NOEL	LD_{50}
Rabbit	0.3 ml saline	3 males/3 females	Not relevant (non-irritant)	Not relevant (non-irritant)
Primary eye irritation				
Species	Vehicle	Sex	NOEL	LD_{50}
Rabbit	Moistened with sterile water	3 males/3 females	Not relevant (non-irritant)	Not relevant (non-irritant)
Acute dermal				
Species	Vehicle	Sex	NOEL	LD_{50} (cfu/animal)
Rabbit	Sterile water	5 males/5 females	Not determined	> 2.3-2.7 x 10^{11}
Acute intravenous				
Species	Vehicle	Sex	NOEL (cfu/ animal)	LD_{50} (cfu/animal)
Rat	Sterile water	3 rats per group/ sacrifice day/ sex	> 9.4 x 10^6	> 9.4 x 10^6

The active substance, *Bacillus subtilis*, has no toxic or clinical effects after oral, intravenous or dermal administration to rats. Very slight irritating effects were recorded after skin exposure and following application to the eye of rabbits but symptoms did not imply a classification according to the relevant EC directive 67/548/EEC. This also applies to the intratracheal challenge, which caused generally minor and mostly short-termed symptoms but no deaths or gross lesions at final necropsy. A skin sensitisation test was performed with the preparation, Serenade™ WP. Based on the sensitising potential of the formulation and the absence of data on skin sensitisation by the active ingredient, QST 713 Technical is classified as a skin sensitiser (R43).

According to the above mentioned results and to the all in all low risk potential of *Bacillus subtilis* further studies concerning the

- Short-term toxicity
- Genotoxicity potential
- Long-term toxicity and carcinogenicity
- Reproductive toxicity
- Teratogenicity potential and
- Neurotoxicity potential

were not performed. However, an additional repeated dose inhalation study is considered necessary by the Rapporteur.

No clinical cases relating to strain QST 713 of *Bacillus subtilis* were reported to occur in the laboratories and production facilities of the applicant. In the literature, incidents of progressive *Bacillus subtilis* infections were only reported for immuno-deficient patients suffering e.g. from leukemia. No specific clinical signs or poisoning symptoms can be attributed to strain QST 713 of *Bacillus subtilis*, accordingly no special therapeutic regimes can be recommended for this non-toxic and non-pathogenic micro-organism.

In the absence of any significant evidence for toxicity, pathogenicity or infectivity of the strain QST 713 of *Bacillus subtilis* in animal studies it is neither possible nor necessary to establish **ADI, AOEL** or **ARfD** values. However, based on the information on the allergic potential of subtilisin produced by *Bacillus subtilis* and dermal effects of the strain QST of *Bacillus subtilis* the requirement of a protective equipment for operators is suggested. In particular with regard to the occurrence of allergies, post registration surveillance should be considered. In addition, results from health and exposure monitoring during production of the product must be submitted since this information may can give indications of possible adverse effects upon repeated exposure.

Bacillus subtilis is a micro-organism which belongs to our natural environment and is generally considered as non-pathogenic to humans in the literature (de BOER & DIDERICHSEN 1991; EPA 1997; FROMMER et al 1989; HARWOOD 1989a). According to the U.S. EPA (1997), *Bacillus subtilis* does not appear to have specialised attachment mechanisms typically found in organisms capable of colonizing the human body. FROMMER et al (1989) state a long history of safe use of *Bacillus subtilis* in enzyme production and that worker protection does not require containment of this micro-organism. Furthermore, this species falls under Class 1 Containment of European Federal Law of Biotechnology (EPA 1997).

Reviewing clinical cases and referring to GRAS petitions, which in no case demonstrated invasive properties of *Bacillus subtilis*, de BOER & DIDERICHSEN (1991) concluded that *Bacillus subtilis* is a safe host for the production of harmless products.

The only secondary metabolite of *Bacillus subtilis* with health concern is the proteinaceous compound subtilisin, which has merely been attributed to allergic reactions of exposed individuals, such as workers in fermentation facilities (EPA 1997).

B.6.6 Step I - Basic acute toxicity studies (preparation) (Annex IIIB 7.1)

The *Bacillus subtilis* (strain QST 713) is formulated as a dried wettable powder. The content of the active substance in QST 713 WP (trade name: Serenade^{TM} WP) is on average 10% by weight, ranging between 7 and 13% to maintain a consistent cell count of 5×10^9 colony forming units (cfu) per g of the product. The main ingredients of the formulated product are kaolin clay and residual fermentation material. Additionally some surfactants and other co-formulants are added.

Acute toxicity tests with QST 713 WP demonstrate that the product is of a low order of acute toxicity to rats by the oral, dermal and respiratory routes, supporting LD_{50} values of >5000 mg/kg bw (~ 2.5×10^{10} cfu/kg bw) following oral and of >2000 mg/kg bw (1×10^{10} cfu/kg bw) following dermal administration. In the acute inhalation test, the highest obtainable aerosol concentration of 0.63 mg/l (~ 5×10^8 cfu/kg bw) caused only clinical symptoms over 2 days. QST 713 WP was non-irritating to the skin and eye of the New Zealand White rabbit. Based on the positive result of the Buehler test and the information of the co-formulants as well as the active ingredient (B.6.5), QST 713 WP should be classified as sensitising so that labelling with the risk phrase R 43 "may cause sensitisation by skin contact" is warranted.

A summary of the results from the acute toxicity studies including irritancy and sensitisation conducted with QST 713 WP is presented in Table B.6.6-1. According to the Directive 78/631/EEC (in combination with the latest classification and labelling guidance under Directive 67/548/EEC; i.e. in the 18th ATP published as Directive 93/21/EEC), a classification/labelling with Xi, R43 (irritant; may cause sensitization by skin contact) is proposed for the product QST 713 WP.

Table B.6.6-1: Summary of acute toxicity studies including irritancy and sensitisation conducted with QST 713 WP

Study type	Species / strain	Results		Reference
LD_{50}, oral; limit test	CD®rats: 5 male / 5 female	NOEL: >5000 mg/kg bw	LD_{50}: >5000 mg/kg bw (~ 2.5×10^{10} cfu/kg bw)	Bellet (1998d) - B.6.6.1 -
LD_{50}, dermal; limit test	New Zealand White rabbits: 5 male / 5 female	NOEL: not determined	LD_{50}: >2000 mg/kg bw (~ 1×10^{10} cfu/kg bw)	Bellet (1998e) - B.6.6.3 -
LC_{50}, inhalation; limit test	SD®rats: 5 male / 5 female	NOEL: not determined	>0.63 mg/l air (4h); maximum aerosol concentration (~ 5×10^8 cfu/kg bw)	Douds (1998) - B.6.6.2 -
Skin irritation	New Zealand White rabbits: 3 males / 3 females	non-irritant		Bellet (1998f) - B.6.7.1 -
Eye irritation	New Zealand White rabbits: 3 males/ 3 females	non-irritant		Bellet (1998g) - B.6.7.2 -
Skin sensitisation	Hartley Guinea pigs 10 male / 10 female	sensitising		Bellet (1998h) - B.6.7.3 -

B.6.6.1 Acute oral toxicity (Annex IIIB 7.1.1)

Report: Bellet, E. (1998d): Acute oral toxicity exposure study in rats with QST 713 WP; Chrysalis Preclinical Services Corporation, Olyphant, U.S.A.; unpublished; Study No. 0402XA54.001; dates of experimental work: June 16, 1998 – June 30, 1998

Test Material: Wettable powder formulation consisting of *Bacillus subtilis* (QST 713 WP, Lot No. 32.38.3B)

Test Animals: Sprague Dawley Rat – Crl:CD®(SD)BR

GLP: Yes (self certification by the laboratory)

Test Method: EPA-Pesticide Assessment Guidelines, Subdivision F (No. 81-1); Corresponds to EEC B1 - Directive 92/69/EEC (limit-test, one dose level), and to OECD guideline 401 (applying to chemical substances)

Deviations: Any data supplied by the sponsor AgraQuest, such as identity, strength, purity, stability and composition, were not subjected to confirmatory analysis by the testing facility.

Acceptability: The study is considered to be acceptable.

Material and Methods:
The test substance was a wettable powder formulation consisting of *Bacillus subtilis* (QST 713 WP, Lot No. 32.38.3B). In the limit test, the test substance was suspended in distilled water and orally administered to CD®rats (5 male, 5 female) at a dose of 5000 mg/kg of body weight (~ 2.5 x 10^{10} cfu/kg bw). Observations were recorded 1 and 4 h after dosing and once daily through Day 15, when rats were sacrificed for necropsy.

Findings:
QST 713 WP had no toxic or pathogenic effect on rats. No deaths occurred and no clinical signs were observed during the 15-day observation period. Necropsy findings were normal, no visible lesions were observed in any of the test animals. Body weights of all animals increased during the test.
Oral NOEL: > 5000 mg/kg body weight (~ 2.5 x 10^{10} cfu/kg bw).
The LD_{50} exceeds 5000 mg/kg bw as well, but could not be calculated because no mortality occurred in this limit test.

Conclusion:
The oral NOEL value of 5000 mg/kg bw exceeds 2.5 times the limit value for harmful substances. Therefore the product QST 713 WP can be evaluated as non-toxic by the oral route and no classification and labelling is required.

B.6.6.2 Acute inhalation toxicity (Annex IIIB 7.1.2)

Report: Douds, George A. (1998): An acute whole-body inhalation toxicity study in rats with QST 713 WP; Springborn Laboratories Inc. (SLI) Spencerville, Ohio, U.S.A.; unpublished; Study No. 3474.1; dates of experimental work: Aug. 3, 1998 – Aug. 17, 1998

Test Material: Wettable powder formulation consisting of *Bacillus subtilis* (QST 713 WP, Lot No. 32.38.10B)

Test Animals: Sprague Dawley®SD®-rats

GLP: Yes (self certification by the laboratory)

Test Method: EPA-Pesticide Assessment Guidelines, Subdivision F (No. 81-3); Corresponds to OECD guideline 403 (limit test) (applying to chemical substances)

Deviations: Any data supplied by the sponsor AgraQuest, such as identity, strength, purity, stability and composition, were not subjected to confirmatory analysis by the testing facility.

Acceptability: The study is considered to be acceptable.

Material and Methods:

The test substance was a wettable powder formulation consisting of *Bacillus subtilis* (QST 713 WP, Lot No. 32.38.10B). In the limit test, the test substance (5×10^9 cfu/g) was mixed with deionized water and individually caged SD®rats (5 male, 5 female) were exposed to a time-weighted average aerosol concentration of 0.63 mg/l of the product – the maximum obtainable aerosol concentration maintaining a median aerodynamic particle size < 4.0 µ. Assuming a respiratory volume in rats of 45 l/kg bw/h, during the 4 h of the test 180 l/kg bw were inhaled, corresponding to approximately 5×10^8 cfu/kg bw. (0.63 mg/l x 180 l/kg bw = 113 mg/kg bw). Observations were recorded daily and rats were weighed weekly through Day 14, when rats were sacrificed for necropsy.

Findings:

No mortality occurred. Primarily clinical symptoms appeared on Day 0 up to Day 2, later most rats were free from symptoms (7 out of 10). Symptoms included: wobbly gait, temporary salivation, apparent hypothermia, urine stain, dark material around the facial area, breathing abnormalities, decreased defecation, decreased food consumption, decreased activity and a hunched posture. 2 animals exhibited continued eye (cornea shape and opacity) reactions, up to Day 14.

Body weight alterations were not assigned to treatment with the test substance.

Necropsy findings on Day 14 were normal.

LC_{50}: >0.63 mg/l aerosol (~ 5×10^8 cfu/kg bw) (= maximum obtainable aerosol concentration; gravimetrically determined).

Discussion:
No mortality occurred when QST 713 WP is inhaled by rats at the tested concentration of 0.63 mg/l aerosol (~ 5 x 10^8 cfu/kg bw). Only some clinical symptoms were observed over two days after the treatment. However the tested aerosol concentration did not cover the limit values set for a classification as toxic or harmful substance which are 1 mg and 5 mg/l air respectively. Since the formulants are non-toxic, the corresponding intratracheal toxicity study conducted with the technical product can be referred to (see B.6.1.1.2): the LD_{50} exceeded 1.2 x 10^8 cfu/rat, corresponding to ~ 20 mg of formulated product per rat or ~ 100 mg product/kg bw (i.e ~ 5 x 10^8 cfu/kg bw). Thus in both tests the tested amounts of the bacillus were in the same range. Considering the difficulties to create a fine aerosol of QST 713 TP, failing to establish a concentration of only 1 mg/l air it appears to be unrealistic to have effective aerosol concentrations under field conditions. Thus, any toxic inhalation effects are not anticipated.

Conclusion:
QST 713 WP did not cause mortality when inhaled by rats at the tested concentration of 0.63 mg/l aerosol (= maximum obtainable aerosol concentration). Only some primarily clinical symptoms were observed over two days. Thus no classification and labelling is required.

B.6.6.3 Acute percutaneous toxicity (Annex IIIB 7.1.3)

Report:	Bellet, E. (1998e): Acute exposure dermal toxicity in rabbits with QST 713 WP; Chrysalis Preclinical Services Corporation , Olyphant, U.S.A.; unpublished; Study No. 0422XA54.001; dates of experimental work: June 17, 1998 – July 1, 1998
Test Material:	Wettable powder formulation consisting of *Bacillus subtilis* (QST 713 WP, Lot No. 32.38.3B); test article was applied neat, moistened with ~ 1.5 ml of distilled water respectively
Test Animals:	New Zealand White (HM (NZW) fBR) rabbits
GLP:	Yes (self certification by the laboratory)
Test Method:	Corresponds to EPA Microbial Pesticide Test Guidelines OPPTS 885.3100; Corresponds to EEC B3 - Directive 92/69/EEC (limit-test, one dose level), and to OECD guideline 402 (applying to chemical substances)
Deviations:	Any data supplied by the sponsor AgraQuest, such as identity, strength, purity, stability and composition, were not subjected to confirmatory analysis by the testing facility.
Acceptability:	The study is considered to be acceptable.

Material and Methods:
The test substance was a wettable powder formulation consisting of *Bacillus subtilis* (QST 713 WP, Lot No. 32.38.3B). The test article (5×10^9 cfu/g) was moistened with approximately 1.5 ml of distilled water and was administered once to the skin of 5 male and 5 female New Zealand White (HM (NZW) fBR) rabbits at 2000 mg/kg bw (~ 1×10^{10} cfu/g) for a 24 h exposure-period (limit test). Observations and recording of clinical signs were performed at unwrapping and daily thereafter through Day 15 (terminal necropsy).

Findings:
No deaths occurred during the 14-day observation period. All animals gained weight and no clinical signs were observed during the study. Only abnormal posture was observed in one animal on Day 3-6. Skin reactions at the application site (erythema, edema, necrosis, fissuring and/or sloughing of the skin) were observed in all animals. At terminal necropsy no visible lesions were observed.

Conclusion:
QST 713 WP did not act toxic following dermal exposure. Only skin reactions at the application site were observed The dermal LD_{50} was estimated to be >2000 mg/kg bw. Therefore, no classification of the product QST 713 WP is required.

B.6.7 Step II - Additional acute toxicity studies (preparation) (Annex IIIB 7.2)

B.6.7.1 Skin irritation (Annex IIIB 7.2.1)

Report: Bellet, E. (1998f): Primary dermal irritation in rabbits with QST 713 WP; Chrysalis Preclinical Services Corporation , Olyphant, U.S.A.; unpublished; Study No. 0420XA54.003; dates of experimental work: June 16, 1998 – June 19, 1998

Test Material: Wettable powder formulation consisting of *Bacillus subtilis* (QST 713 WP, Lot No. 32.38.3B); test article was applied neat, moistened with 0.2 ml saline (Baxter, Lot No. C362293) respectively

Test Animals: New Zealand White (HM (NZW) fBR) rabbits

GLP: Yes (self certification by the laboratory)

Test Method: Dose selection and route of administration according to EPA Pesticide Assessment Guidelines Subdivision F (No. 81-5);
Corresponds to EEC B4 – Directive 92/69/EEC, and to OECD guideline 404 (applying to chemical substances)

Deviations: Any data supplied by the sponsor AgraQuest, such as identity, strength, purity, stability and composition, were not subjected to confirmatory analysis by the testing facility.

Acceptability: The study is considered to be acceptable.

Material and Methods:
The test substance was a wettable powder formulation consisting of *Bacillus subtilis* (QST 713 WP, Lot No. 32.38.3B). Respectively 500 mg of the test article was moistened with 0.2 ml saline (Baxter, Lot No. C362293) and applied to the shaved skin of 3 male and 3 female rabbits (HM: NZW fBR). The area was covered with a gauze patch secured with a semi-occlusive dressing, for a 4 h period. At the end of the 4 h exposure period, the treated sites were unwrapped and residual test article was removed with water and gauze. Subsequent observations were recorded at 30-60 minutes, 24 , 48 and 72 h following unwrapping. Signs for dermal irritation were scored according to Draize.

Findings:
QST 713 WP (~ 500 mg) caused very slight erythema and/ or very slight edema at rabbit skin (maximum Draize score: 1). The skin recovered from symptoms within the observation period of 72 h. No other dermal signs were noted.

Conclusion:
QST 713 WP proved to be only very slight irritant after 4 h exposure. No classification of the product is required.

B.6.7.2 Eye irritation (Annex IIIB 7.2.2)

Report:	Bellet, E. (1998g): Primary eye irritation in rabbits with QST 713 WP; Chrysalis Preclinical Services Corporation , Olyphant, U.S.A.; unpublished; Study No. 0421XA54.003; dates of experimental work: June 26, 1998 – June 29, 1998
Test Material:	Wettable powder formulation consisting of *Bacillus subtilis* (QST 713 WP, Lot No. 32.38.3B); preparation: test article was applied as received (powder), packed into a 1cc syringe to a volume of 0.1 ml.
Test Animals:	New Zealand White (HM (NZW) fBR) rabbits
GLP:	Yes (self certification by the laboratory)
Test Method:	Dose selection according to EPA Pesticide Assessment Guidelines Subdivision F (No. 81-4), partly also applying to scale for scoring ocular lesions. Tabulating of test article according to Addendum 2 on EPA Pesticide Assessment Guidelines – Eye Irritation (1988); Corresponds to EEC B5 - Directive 92/69/EEC, and to OECD guideline 405 (applying to chemical substances)
Deviations:	Any data supplied by the sponsor AgraQuest, such as identity, strength, purity, stability and composition, were not subjected to confirmatory analysis by the testing facility.
Acceptability:	The study is considered to be acceptable.

Material and Methods:
The test substance was a wettable powder formulation consisting of *Bacillus subtilis* (QST 713 WP, Lot No. 32.38.3B). Approximately 100mg of the test article was applied as received (powder; packed into a 1cc syringe to a volume of 0.1 ml) into the conjunctival sac of the right eye of each of 3 male and 3 female New Zealand White (HM (NZW) fBR) rabbits. Observations and recording of ocular irritation were performed according to Draize at 1, 24, 48 and 72 h post dose administration.

Findings:
Slight irritation of the conjunctivae (predominantly redness, infrequently chemosis and/or discharge) was observed in all animals at 1 h post-dose. Redness persisted up to 48 h in 50% of test animals. All symptoms ceased within 72 h post-dose. The calculation gives following values, referring to each symptom separately:

Symptom	**Mean score value** (24, 48,72 h)	Classification
Cornea opacity	0	none
Iris lesions	0	none
Redness of conjunctivae	0.4	none
Chemosis of conjunctivae	0	none

Conclusion:
QST 713 WP is determined to be practically non-irritant and no classification and labelling is required.

B.6.7.3 Skin sensitisation (Annex IIIB 7.2.3)

Report: Bellet, E. (1998h): Delayed contact hypersensitivity in Guinea Pigs with QST 713 WP (Buehler Method); Chrysalis Preclinical Services Corporation; Olyphant, U.S.A.; unpublished; Study No. 0424XA54.001; dates of experimental work: June 23, 1998 – July 31, 1998

Test Material: Wettable powder formulation consisting of *Bacillus subtilis* (QST 713 WP, Lot No. 32.38.3B); test article was applied neat, moistened with ~ 0.3 ml of distilled water respectively

Test Animals: Hartley Guinea Pigs (Elm: HA)

GLP: Yes (self certification by the laboratory)

Test Method: EPA (FIFRA) Subdivision F guideline No. 81-6 (Buehler Method); Corresponds to EEC B6 - Directive 92/69/EEC, and to OECD guideline 406 (applying to chemical substances)

Deviations: Any data supplied by the sponsor AgraQuest, such as identity, strength, purity, stability and composition, were not subjected to confirmatory analysis by the testing facility.

Acceptability: The study is considered to be acceptable.

Material and Methods:
The test substance was a wettable powder formulation consisting of *Bacillus subtilis* (QST 713 WP, Lot No. 32.38.3B). Based on the results of a primary irritation screen where no effects were seen up to concetrations of 100%, the test article was administered at 100% (neat) for induction (3 x), challenge and rechallenge (one week after challenge at a previously untreated site) to the skin of 10 male and 10 female Hartley Guinea Pigs (6 h occluded dermal application). The test article was moistened with respectively ~ 0.3 ml of distilled water. DNCB (1-chloro-2,4-dinitrobenzene) served as positive control (0.3% in ethanol for induction; 0.2% in acetone for challenge). All animals were observed for dermal irritation and scored at 24 and 48 h after each exposure period.

Findings:
QST 713 WP (100%) did elicit a mild delayed contact hypersensitivity response in guinea pigs after challenge and rechallenge (Table B.6.7-1). The maximum irritation symptom was moderate erythema, most responding animals exhibited only slight to moderate erythema or even less intense symptoms. After challenge 30% of the test animals showed a response at score 1 and 20% at score 2 (at 48h), after rechallenge 5% of the tested guinea pigs reacted positively with score 1 resp. 5% with score 2 (at 48h).

Table B.6.7-1 Percentage of animals with scores after 24 and 48 h following challenge and rechallenge with QST 713 WP

Challenge												
Group	Induction	Challenge	24 h-scores					48 h-scores				
			0	± [1)]	1	2	3	0	± [1)]	1	2	3
Positive control	0.3% DNCB in 80% ethanol	0.2% DNCB in acetone	0 80	0 0	0 10	50 10	50 0	0 80	0 20	33 0	50 0	17 0
Vehicle control	Distilled water	Distilled water QST 713 WP	100	0	0	0	0	100	0	0	0	0
Test article	QST 713 WP	Distilled water QST 713 WP	100 50	0 30	0 10	0 10	0 0	100 30	0 20	0 30	0 20	0 0
Rechallenge												
Test article	QST 713 WP	QST 713 WP	40	25	25	10	0	70	20	5	5	0

[1)] Slightly patchy erythema

Discussion:
The positive results (after 24h: 20%; after 48h: 50%) from the animal test provide evidence for a skin sensitising potential of QST 713 WP. Classification and labelling with the risk phrase R 43 (may cause sensitisation by skin contact) is required accordingly. Also an allergic potential of subtilisin produced by *Bacillus subtilis* is described in humans (B.6.5). Nevertheless, a sensitization study with QST 713 Technical is not available.
Additionally, for the product QST 713 WP, due to the content of the preservative Proxel and therefore 1,2-benzisothiazolin (B.I.T.), a potential to cause skin sensitisation cannot be excluded.
On the basis of the only very slight sensitization effects in a rechallenge after 48 hours, the notifier, however, had aproposed not to classify neither the product QST 713 WP nor the active ingredient (QST 713 Technical) as a sensitiser.

Conclusion:
Based on the positive result of the Buehler test and with regard to the available information on the co-formulants as well as on the active ingredient, QST 713 WP should be classified as

sensitising. Thus, labelling with the risk phrase R 43 ”may cause sensitisation by skin contact” is warranted.

B.6.8 Data on exposure(Annex IIIB 7.3)

QST 713 WP (Serenade WP) is formulated as a dried wettable powder. The content of the active substance (~ 5 x 10^{10} cfu/g) is on average 10% by weight, ranging between 7 and 13% to maintain a consistent cell count of 5 x 10^{9} colony forming units (cfu) of *Bacillus subtilis* per g of the product.
QST 713 WP is used as a fungicide and bactericide. The maximum dosage rate used for control of diseases is 15.0 kg product per ha, i.e. max. 1.5 kg as/ha in orchards and 12.0 kg product per ha, i.e. max. 1.2 kg as/ha in lettuce, respectively. The relevant modes of application to be considered are:

- Field crop tractor-mounted sprayer (lettuce) – (FCTM),
  Use rate: 1.2 kg as/ha; treated area per day: 20 ha;
- High crop tractor-mounted boom sprayer (orchards, vines) - (HCTM),
  Use rate: 1.5 kg as/ha; treated area per day: 8 ha;
- High crop hand-held equipment (orchards, vines) - (HCHH),
  Use rate: 1.5 kg as/ha; treated area per day: 1 ha.

The estimated exposures have been calculated according to the German model (Uniform Principles for Safeguarding the Health of Applicators of Plant Protection Products; Uniform Principles for Operator Protection; Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft, Berlin-Dahlem, no. 277, 1992) using the maximum application rates.

Table B.6.8-1: Estimated operator exposure (German model: no PPE)

	Estimated exposure					
	Dermal		**Inhalation**		**Total**	
	mg/person/d	**mg/kg bw/d***	**mg/person/d**	**mg/kg bw/d***	**mg/person/d**	**mg/kg bw/d***
FCTM	192.96	2.76 (~1.5 x 10^{8} cfu per kg bw/d)	1.704	0.02 (~ 1 x 10^{6} cfu per kg bw/d)	194.664	2.78 (~1.5 x 10^{8} cfu per kg bw/d)
HCTM	210.00	3.00 (~ 1.5 x 10^{8} cfu per kg bw/d)	1.056	0.02 (~ 1 x 10^{6} cfu per kg bw/d)	211.056	3.02 (~1.5 x 10^{8} cfu per kg bw/d)
HCHH	135.60	1.94 (~ 1 x 10^{8} cfu per kg bw/d)	1.650	0.02 (~ 1 x 10^{6} cfu per kg bw/d)	137.250	1.96 (~ 1 x 10^{8} cfu per kg bw/d)

*body weight: 70 kg

As it can be taken from Table B.6.8-1, the estimated total exposure is in the range of 2-3 mg/kg bw/d (without PPE) corresponding to 1-1.5 x 10^8 cfu/kg bw/d. The predominant part is the dermal exposure, although a relevant dermal absorption of the bacillus is not expected. Additionally, in the acute dermal studies, ~ 2.5 x 10^{11} cfu/animal (QST 713 Technical on rabbits) and ~ 1 x 10^{10} cfu/animal (2000 mg/kg bw QST 713 WP on rabbits) showed only local effects.
Only about 1/100 of the estimated total exposures would be inhaled. The estimated inhalation exposures of approximately 0.02 mg/kg bw/d in all cases correspond to ~ 1 x 10^6 cfu/kg bw/d respectively. To roughly asses these values, only the results from the acute toxicity studies with the active ingredient (QST 713 Technical) and with the formulation (QST 713 WP) can be noted. Thus an oral NOAEL was found at 5 x 10^8 cfu/kg bw (QST 713 Technical on rats). In the respective test with QST 713 WP, the tested dose of 5000 mg/kg bw/d (corresponding to 2.5 x 10^{10} cfu/kg bw) shows also no effects. Clinical effects but no death were seen in the intratracheal study with QST 713 Technical and in the inhalation study with QST 713 WP where 5 x 10^8 cfu/kg bw were applied on rats, respectively. In the study with the product the tested average aerosol concentration of 0.63 mg/l (corresponding to approximately 5 x 10^8 cfu/kg bw) was the maximum obtainable aerosol concentration maintaining a median aerodynamic particle size < 4.0 µ (to guarantee the respirability of the test substance).
Thus taking the biological properties of *Bacillus subtilis* into consideration and the very low acute toxicity of active substance and the tested formulation QST 713 WP, no toxic effects are expected to occur after exposure to QST 713 WP for operators, workers or bystanders.

B.6.9 Available toxicological data relating to non-active substances (Annex IIIB 7.4)

Beside the active micro-organism, QST 713 WP (Serenade^{TM} WP) mainly contains kaolin clay and residual fermentation broth. Additionally the formulation contains various co-formulants known for common use in food, pharmaceutical and cosmetic industrie. Material Safety Data Sheets (MSDS) for all the auxiliaries which are contained in small amounts of <5% altogether are submitted and the possibly toxic properties are covered by the studies with the preparation. Nevertheless, QST 713 WP contains the preservative Proxel and therefore 1,2-benzisothiazolin (B.I.T.). There is evidence that B.I.T. may cause skin sensitisation at levels above 500 ppm (0.05%) and that products containing more than 500 ppm of B.I.T. should be labelled as potential skin sensitisers.

Thus, on the basis of all available data, a skin sensitisation potential cannot be excluded for the product QST 713 WP and classification and labelling should be proposed accordingly.

B.6.10 Supplementary studies for combination of plant protection products (Annex IIIB 7.5)

The applicant does not recommend tank mixes with specific plant protection products, but stated that tank mixes with chemical or other biological plant protection products may be used provided that experience has shown physical compatibility and efficacy. No toxicological studies have been performed using combinations of QST 713 WP with other plant protection products.

B.6.11 Summary and evaluation of health effects (Annex IIIB 7.6)

Table B.6.11-1: Summary of acute toxicity studies including irritancy and sensitisation conducted with QST 713 Technical and QST 713 WP

Study type; strain	Test substance	Dose	Results	Reference
LD_{50}, i.v., rat; limit test	QST 713 Techn.	9.4 x 10^6 cfu/animal	No effects at ~ 5 x 10^7 cfu/kg bw	Harrington (1998c) B.6.3.1
LD_{50}, oral, rat; limit test	QST 713 Techn.	(1 ml/animal) 1.13 x 10^8 cfu/animal	No effects at ~ 5 x 10^8 cfu/kg bw	Harrington (1998a) B.6.1.1.1
LD_{50}, oral, rat; limit test	QST 713 WP	5000 mg/kg bw (~ 2.5 x 10^{10} cfu/kg bw)	No effects at ~ 2.5 x 10^{10} cfu/kg bw	Bellet (1998d) B.6.6.1
LD_{50}, dermal, rabbit; limit test	QST 713 Techn.	2.3-2.7 x 10^{11} cfu/animal	Only local effects at ~ 2.5 x 10^{11} cfu/kg bw	Finley (1998) B.6.2.1
LD_{50}, dermal, rabbit; limit test	QST 713 WP	2000 mg/kg bw (~ 1 x 10^{10} cfu/kg bw)	Only local effects at ~ 1 x 10^{10} cfu/kg bw	Bellet (1998e) B.6.6.3
LD_{50}, intratracheal, rat; limit test	QST 713 Techn.	1.2 x 10^8 cfu/animal	Only clinical effects at ~ 5 x 10^8 cfu/kg bw (clearence: >100 d)	Harrington (1998b) B.6.1.1.2
LC_{50}, inhalation, rat; limit test	QST 713 WP	0.63 mg/l air (4h); (~ 5 x 10^8 cfu/kg bw)	Only clinical effects at ~ 5 x 10^8 cfu/kg bw (max. attainable conc.)	Douds (1998) B.6.6.2
Skin irritation, rabbit	QST 713 Techn.	non-irritant (very slight erythema)		Bellet (1998b) B.6.1.1.4
Skin irritation, rabbit	QST 713 WP	non-irritant (very slight erythema/edema)		Bellet (1998f) B.6.7.1
Eye irritation, rabbit	QST 713 Techn.	non-irritant (very slight effects)		Bellet (1998c) B.6.1.1.5
Eye irritation, rabbit	QST 713 WP	non-irritant		Bellet (1998g) B.6.7.2
Skin sensitisation, Guinea pigs; Buehler test	QST 713 WP	sensitising		Bellet (1998h) B.6.7.3

The acute toxicity tests with the product QST 713 WP as well as the active ingredient QST 713 Technical (Table B.6.11-1) demonstrate that the product is of a low order of acute toxicity by the oral, dermal and respiratory routes, supporting LD_{50} values of >5000 mg/kg bw (~ 2.5 x 10^{10} cfu/kg bw) following oral administration to rats and of >2000 mg/kg bw (1 x 10^{10} cfu/kg bw) following dermal administration to rabbits. In the acute inhalation test on rats, the highest obtainable aerosol concentration of 0.63 mg/l (~ 5 x 10^8 cfu/kg bw) caused only clinical symptoms over 2 days. QST 713 WP was non-irritating to the skin and eye of the New Zealand White rabbit. Based on the positive result of the Buehler test and the information of the co-formulants as well as the active ingredient (B.6.5), QST 713 WP should be classified as sensitising so that labelling with the risk phrase R 43 ”may cause sensitisation by skin contact” is warranted.

The potential exposure for operators was estimated for the intended uses of QST 713 WP (Serenade WP; 5 x 10^9 cfu/g). The calculation using the German model and data for worst case conditions results in estimated total exposures in a range of 2-3 mg as/kg bw/d (without PPE) corresponding to 1-1.5 x 10^8 cfu/kg bw/d. The dermal exposure is the predominant part of the estimated exposure, but not a relevant dermal absorption of the bacillus is to expect. Only about 1/100 of the values would be inhaled (~ 1 x 10^6 cfu/kg bw/d).

In relation to the results of the available acute toxicity studies, sufficient margins of safety are to be seen although not in all studies "No Effect Levels" were demonstrated and in the intratracheal study with the active ingredient, the complete clearance from all tissues was estimated to take a time of >100 days (no evidence of germination or vegetative growth). Short term toxicity studies are not available.
Basing on the estimated exposures in relation to the available toxicity data and according to the biological properties of *Bacillus subtilis strain QST 713*, harmful effects on the health of operators, workers or bystanders are not to be expected when the products are used in accordance with good plant protection practice. Nevertheless, due to the possible sensitising potential of the active ingredient and the sensitising properties of the product, suitable personal protective equipment is necessary, especially when handling the undiluted product

B.6.12 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[6]
AIIB-5.1	Frommer, W. et al.	1989	Safe biotechology. III. Safety precautions for handling microorganisms of different risk classes. not GLP, published Appl. Microbiol. Biotechnol., 30, 1989, 541-552 TOX2000-1215	N	-
AIIB-5.1.1.1	Gingras, B.A.	1998	Sensitivity of detection of bacillus subtilis strain QST 713 for toxicity/pathogenicity testing in rats. L08726 SN3 GLP, unpublished TOX2000-1207	Y	QST
AIIB-5.1.1.1	Harrington, K.A.	1998	Toxicity/pathogenicity testing of QST 713 following acute oral challenge in rats. L08726 SN4 GLP, unpublished TOX2000-1206	Y	QST

[6] Only notifier listed

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[6]
AIIB-5.1.1.4	Harrington, K.	1998	Toxicity/pathogenicity testing of QST 713 following acute intatracheal challenge in rats. . L08726 SN6 GLP, unpublished TOX2000-1208	Y	QST
AIIB-5.1.1.3; AIIB-5.1.1.6	Findlay, J.	1998	Acute dermal toxicity/pathology study of QST 713 in rabbits. L08726SN7 GLP, unpublished TOX2000-1218	Y	QST
AIIB-5.1.1.6	Mallory, V.T.	1998	Primary eye irritation in rabbits with QST 713 TP. 0421XA054.004 GLP, unpublished TOX2000-1211	Y	QST
AIIB-5.1.1.6	Mallory, V.T.	1998	Primary dermal irritation in rabbits with QST 713 TP. 0420XA054.004 GLP, unpublished TOX2000-1210	Y	QST
AIIB-5.1.2	Harwood, C.R.	2000	Introduction to the biotechnology of bacillus. not GLP, published Bacillus, The university of Newcastle upon tyne, UK, Plenum Press, 1-4 TOX2000-1214	N	-
AIIB-5.1.2.2	Harrington, K.A.	1998	Toxicity/pathogenicity testing of QST 713 following acute intravenous challenge in rats. L08726SN5 GLP, unpublished TOX2000-1219	Y	QST
AIIB-5.2.3	Taira, S., Jalonen, E., Paton, J.C., Sarvas, M. and Runeberg-Nyman, K.	1989	Production of pneumolysin, a pneumococcal toxin, in bacillus subtilis. not GLP, published Gene, 77, 1989, 211-218 TOX2000-1213	N	-

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[6]
AIIB-5.1.3; AIIB-5.4.1; AIIB-5.4.2	Anonym	1997	Final decision document: TSCA section 5(H)(4) exemption for bacillus subtilis. not GLP, published EPA, 1997 TOX2000-1216	N	-
AIIB-5.1; AIIB-5.4.3	Ihde, D.C. and Armstrong, D.	1973	Clinical spectrum of infection due to bacillus species. not GLP, published The American Journal of Medicine., 55, 1973, 839-845 TOX2000-1217	N	-
AIIB-5.1; AIIB-5.4.1; AIIB-5.4.3; AIIB-5.4.4	Sietske de Boer, A. and Dide-richsen, B.	1991	On the safety of bacillus subtilis and b. amylo-liquefaciens: a review. not GLP, published Appl. Microbiol. Biotechnol., 36, 1991, 1-4 TOX2000-1212	N	-
AIIIB-7.1.1	Bellet, E.	1998	Acute oral exposure toxicity study in rats with QST 713 WP. 0402XA54.001 GLP, unpublished TOX2000-1199	Y	QST
AIIIB-7.1.2	Douds, G.A.	1998	An acute whole-body inhalation toxicity study in rats with QST 713 WP. 3474.1 GLP, unpublished TOX2000-1200	Y	QST
AIIIB-7.1.3	Bellet, E..	1998	Acute exposure dermal toxicity in rabbits with QST 713 WP. 0422XA54.001 GLP, unpublished TOX2000-1201	Y	QST
AIIIB-7.2.1	Bellet, E.	1998	Primary dermal irritation in rabbits with QST 713 WP. 0420XA54.003 GLP, unpublished TOX2000-1202	Y	QST

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[6]
AIIIB-7.2.2	Bellet, E..	1998	Primary eye irritation in rabbits with QST 713 WP. 0421XA54.003 GLP, unpublished TOX2000-1203	Y	QST
AIIB-5.1.1.7; AIIIB-7.2.3	Bellet, E..	1998	Delayed contact hypersensitivity in guinea pigs with QST 713 WP (Buehler Method). 0424XA54.001 GLP, unpublished TOX2000-1204	Y	QST
AIIIB-2.6; AIIIB-4.4; AIIIB-7.5	Campbell, B., Cunningham-Hilbig, L.	2000	Serenade WP Tank Mix compability interim Report. not GLP, unpublished PHY2000-725	Y	QST
AIIB-5.5; AIIIB-7.6	Thobor, C.	2000	Bacillus subtilis (150g/kg technical powder) & Serenade tm WP (AI: 100g/kg, formulation: wettable powder) - Summary of mammalian toxicity, pathogenicity and infectivity, exposure risk assessments and overall evaluation. AB981202-EU09/01 not GLP, published TOX2000-1205	Y	-

Codes of owner

QST: AgraQuest, Inc.

Annex B

Bacillus subtilis strain QST 713

B-7: Residue data

B.7 Residue data

B.7.1 Persistence and likelihood of multiplication of the active substance in or on crops, feedingstuffs or foodstuffs (Annex IIB 6.1; Annex IIIB 8)

Persistance and likelihood of multiplication in or on crops, feedingstuffs or foodstuffs

The following statement on the potential role and significance of any residues of *B. subtilis* is based on reported characteristics of this species, as uniformly found in the scientific literature, under consideration of the envisaged application and relevant properties of strain QST 713.

Strain QST 713 of *B. subtilis* is intended to be applied onto the foliage. Regarding the intended fields of use residues of *B. subtilis* on **leaf surfaces** are associated with the establishment of colonisation, the prime phenomenon of this contact-biofungicide and bactericide. Colonisation of treated foliage provides a protective layer and basically is involved in the mode of action of *B. subtilis* against pathogen attack.

Campbell (1989, BMF 2000-100) addresses to the problems involved in foliar application of biocontrol agents, including adhesion of preparations to the mainly hydrophobic leaf surface and the usually unfavourable environmental conditions restricting microbial growth (and explaining the generally slight occurrence of growing saprophytic bacteria on the leaf surface and the low covering percentage (<1 %) of micro-organisms on the leaf surface of temperate plants). In addition, *B. subtilis* cells will stop growth after depletion of organic matter supply (EPA, 1997), e.g. the fungal pathogen.
Therefore, the protective layer of *B. subtilis* cells colonising the leaf surface will not maintain stability or persistence for long in this stressed micro-habitat.
The applicant conducted a study on longevity of QST 713 strain of *B. subtilis* on pepper leaf surface under greenhouse conditions, applied at a rate of 6.7 kg/ha formulated product (see Yuan and Heins, 2000): an initial increase in colony forming units (cfu) was followed by a sharp decline in cfu-counts by day 5 to low levels of surviving cells persisting for ~ 3 weeks (see Figure B.7.1-1).

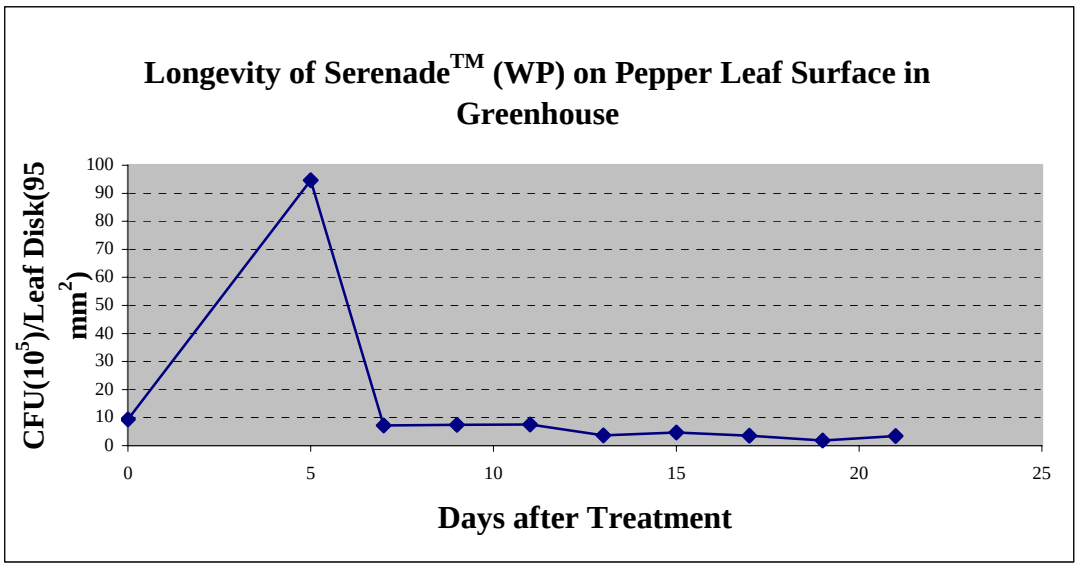

Figure B.7.1-1 Time course of cfu-counts of *B. subtilis* strain QST 713 recovered : from pepper leaf surface

The unfavourable environmental conditions prevailing on the leaf surface will not impede the efficacy of QST 713 strain of *B. subtilis* since the preparation will be added several times at appropriate intervals and in sufficient amounts so that long-term survival or long-term colonisation of the foliage is not necessitated – in fact for the induction of systemic resistance in the plant, presenting one mode of action, an initial challenge by a non-pathogenic species may elicit the resistance response (Campbell, 1989, BMF 2000-100).

B. subtilis may form endospores under nutrient shortage and environmental stress. These endospores might possibly be dissipated via wind and water to other environmental compartments (Priest, 1993), where they may contribute to the existent population of *B. subtilis*, e.g.:
Sholberg *et al.* (1995) recovered 95 bacterial isolates from stored apples for screening of biocontrol activity and identified 7 among 30 Bacilli isolates as *B. subtilis* which acted effectively against molds.
With regard to *B. subtilis* cells remaining on harvested fruit the chance of proliferation during processing of raw products (grapes, apples to vine, juice respectively) is not relevant since a) in vine fermentation conditions are unfavourable and b) in juice production microbial contaminants are heat killed (at a processing temperature of ~ 90°C), while conditions do not favour endospore formation.

In this context it has to be considered that *B. subtilis* is a non-pathogenic, ubiquitous micro-organism, primarily a soil inhabitant but has been found in different environmental compartments and media, including the leaf surface and foodstuff (Priest, 1993).

B.7.2 Further information required (Annex IIB 6.2; Annex IIIB 8)

B.7.2.1 Non-viable residues (Annex IIB 6.2.1, Annex IIIB 8)

Not required due to the absence of toxins or other metabolites with undesirable properties.

B.7.2.2 Viable residues(Annex IIB 6.2.2, Annex IIIB 8)

Residue studies were regarded as being dispensable for the following reasons:

- With regard to *B. subtilis* natural global distribution and non-pathogenic character *B. subtilis* cells left on the surface of treated areas or plant products do not imply health or environmental impacts (Boer and Diderichsen, 1991; EPA, 1997).
- *B. subtilis* has been used for enzyme production on a large industrial scale, and is even used for food production without having caused health or environmental hazards or damages (e.g. Priest, 1993).
- *B. subtilis* has no special attachment ability to plants or plant products, i.e. there is no compatibility comparable to host-pathogen interactions (EPA, 1997).
- A plant product (fruit) carrying a layer built up of *B. subtilis* can easily be washed with water prior to consumption or juice production.

B.7.3 Summary and evaluation of residue behaviour (Annex IIB 6.3; Annex IIIB 8)

The natural global distribution of *B. subtilis* and its non-pathogenic character indicate that *B. subtilis* cells of strain QST 713 left on the surface of treated areas or plant products do not imply health or environmental impacts (Boer and Diderichsen, 1991; EPA, 1997). *B. subtilis* does not produce toxins or other metabolites with undesirable properties. The species *B. subtilis* has been used for enzyme production on a large industrial scale, and is even used for food production without having caused health or environmental hazards or damages (e.g. Priest, 1993).

The unfavourable environmental conditions prevailing on the leaf surface and the dependence of *B. subtilis* on organic matter supply are restricting its growth, as shown in the submitted study report (Yuan and Heins, 2000). In addition, in processing of raw products no growth or sporulation of *B. subtilis* is expected to occur.

B.7.4 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[7]
AIIB-6.1	Campbell, R.	1989	Biocontrol on Leaf Surfaces. not GLP, published Cambridge University Press, Cambridge, Department of Botany, Universität of Bristof, 1989, pp. 66-94 RIP2000-2000	Y	-
AIIB-6.1	EPA	1997	Final Decision Document, TSCA Section 5 (H) (4) Exemption for Bacillus Subtilis. not GLP, published RIP2000-2001	N	-
AIIB-6.1	Priest, F.G.	1993	Systematics and Ecology of Bacillus in: Bacillus subtilis and other Gram-Positive Bacteria. not GLP, unpublished RIP2000-2006	Y	QST
AIIB-6.1	Priest, F.G.	1993	Systematics and Ecology of Bacillus in: Bacillus subtilis and other Gram-Positive Bacteria. not GLP, unpublished RIP2000-2002	Y	QST
AIIB-6.1	Yuan, C.; Heins, S.	2000	Longevity Study of Serenade (QST713) on Pepper Leaf Surface in Greenhouse Conditions. Report No. AQ0032 not GLP, unpublished RIP2000-2003	Y	QST
AIIB-6.2	Boer de A. S.; Diderichsen, B.	1991	On the safety of Bacillus subtilis and B. amyloliquefaciens: a review. not GLP, published Appl. Microbiol. Biotechnol, Vol. 36, 1991, pp.1-4 RIP2000-2005	N	-

[7] Only notifier listed

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[7]
AIIB-6.1; AIIB-6.2	Sholberg, P.L.; Marchi, A.; Bechard, J.	1995	Biocontrol of postharvest diseases of apple using Bacillus spp. isolated from stored apples. not GLP, published Can. J. Microbiol. 41, 1995, pp.247-25 RIP2000-2004	N	-
AIIB-6.1; AIIB-6.2	Yuan, C.; Heins, S.	2000	Longevity Study of Serenade (QST713) on Pepper Leaf Surface in Greenhouse Conditi-ons. Report No. AQ0032 not GLP, unpublished RIP2000-1998	Y	QST

Codes of owner

QST: AgraQuest, Inc.

Annex B

Bacillus subtilis strain QST 713

B-8: Environmental fate and behaviour

B.8 Environmental fate and behaviour

B.8.1 Persistence and multiplication in soil (Annex IIB 7.1.1; Annex IIIB 9)

General remark

The following statement on the fate and behaviour of *B. subtilis* introduced into the environment is based on reported characteristics of this species, as uniformly found in the scientific literature, under consideration of the envisaged application and relevant properties of strain QST 713.
On principle, the usually employed model calculations on the persistence of chemical substances are not applicable to an active ingredient being a viable organism. Unlike a chemical substance a micro-organism does not follow first order kinetics in degradation. Therefore, no time-weighted average concentrations can be calculated for the different environmental media.

Beside the active micro-organism, Serenade™ WP contains inert ingredients of the formulation and residuals of the fermentation broth, which are non-toxic and impose no environmental or health risk. Thus, it can be concluded that information about these features given for the active ingredient, *B. subtilis*, are representative for both, the active micro-organism as well as the formulated product.

Persistence and multiplication

With regard to environmental concern of the deliberate release of micro-organisms Glick et al. (1999, BOD2000-1371) state that usually the number of introduced organisms declines rapidly (sometimes after a brief period of proliferation) following application to both the soil microcosms and the field. The authors also say that it is difficult to predict the proliferation of an introduced bacteria and that usually these strains fare less well than indigenous bacteria strains, esp. in case the introduced strains were carrying additional genetic load due to inserted plasmids.

Persistence and multiplication in/ on soil

The phenomenon of fast decreasing vegetative cell numbers is reported for a *B. subtilis* strain introduced into soil (Asaka *et al.*, 1996, BOD2000-1364), while in parallel sporulation increased. After a few days the cell population was shown to be stabilised as endospores. Elsas *et al.* (1986, BOD2000-1365) found a similar population dynamic of *B. subtilis* cells applied to the soil surface at a level of 3,5 x 10^7 cfu/g: an initial decrease in cell numbers was followed by a stabilisation at levels of ~ 3-5 x 10^3 cfu/g. In this study neither the kind of soil (loamy sand and silty loam) nor the rhizosphere of cropped soil had an influence on the dynamics. Moreover, addition of glucose only initially reduced the decline in cell numbers in the loamy sand and later the counts in the glucose-containing loamy sand columns practically equalled those found in the columns without glucose. In studying the plasmid transfer between Bacilli in soil Elsas *et al.* (1987, BOD2000-1367) showed poor survival of vegetative *B. subtilis* cells in non-sterile soil compared to sterile soil or soil amended with bentonite clay. Survival in sterile soil was enhanced by nutrient addition.
Siala and Gray (1974, BOD2000-1369) showed that *B. subtilis* cells added to acid forest soil did not grow unless fungal growth took place and concluded that the prime factor was nutrient

supply; however an indirect effect of altered pH could not be excluded. Phae *et al.* (1990, BOD2000-1368) demonstrated that *B. subtilis* introduced into a thermophilic composting reactor of sewage sludge not only survived but maintained its antifungal potential.
The supply with fresh organic matter appears to be a key parameter for survival of vegetative cells of *B. subtilis* introduced into soil, while the prevailing form in soil appears to be the endospore.
Available information indicates, that application of organic matter, e.g. manure, will enhance growth of existing *B. subtilis* populations. If no nutrient are supplied the cells will produce endospores.

B. subtilis is an autochthonous soil micro-organism. Strain QST 713 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. As vegetative growth declines as the nutrient source declines this species does not seem to compete well for limited resources and *B. subtilis* populations will be subject to competition in the natural micro-flora (Campbell, 1989, BOD2000-1372) on ecological basics.
Since *B. subtilis*, including this strain, is facultative anaerobic or micro-aerophilic, growth will prevail in the superficial, aerated soil layer. Translocation of *B. subtilis* into deeper soil layers has been shown to occur at low levels (Elsas *et al.*, 1986, BOD2000-1365).
With regard to the intended fields of use (vineyards, orchards and lettuce fields) most organic matter will be supplied to the soil in orchards in spring, with the end of flowering, and in autumns, at leaf fall. Only in springtime Serenade^{TM} WP applications will coincide with significantly increased organic matter supply to the soil surface.
Finally, introduced *B. subtilis* cells are not expected to exceed the natural level permanently.

Predicted load of colony forming units (cfu) on treated areas:

Serenade^{TM} WP is applied to the foliage at a rate of 15 kg/ha in maximum per treatment. An amount of 15 kg/ha corresponds to 7.5×10^{13} cfu/ha. Assuming the whole amount would reach the soil surface uniformly, the resulting surface load would be approximately 7.5×10^{5} cfu/cm^2 per treatment. After maximal 16 applications in orchards the resulting surface load would be approximately 1.2×10^{7} cfu/cm^2.
Employing a more realistic scenario under consideration of drift results in even lower levels of surface load: In consideration that a rate of 50 % of the applied amount of product will reach the soil surface one square cm of surface will receive a theoretical load of 6×10^{6} cfu. The maximal predicted environmental concentration would be 8×10^{5} colony forming units per gram soil (Table B.8.1-1).
This still overestimated value can be regarded as low in view of the generally high population densities of Bacilli, which occur at levels of 10^{6} to 10^{7} cfu/g (EPA, 1997, BOD2000-1366).
Considering the above cited references it can be expected that part of the cells reaching the soil will not survive and the residual cells will form endospores, unless fresh organic matter is supplied.

Table B.8.1-1: Predicted environmental concentration (PEC)

Crop	Product application rate [kg/ha] min max	Number of applications	Interception	PEC [mg product/kg]	Colony forming units [cfu/g soil]
Orchards	5 - 15	16	50 %	max. 160	max. 8×10^5
	5 - 15	1	50 %	max. 10	max. 5×10^4

B.8.2 Persistence and multiplication in water (Annex IIB 7.1.2; Annex IIIB 9)

B. subtilis is frequently occurring in different aquatic environments, as fresh water, estuarine and coastal waters, and endospores have been detected in sediments and even in the open ocean (Priest, 1993, BOD2000-1370). However, *B. subtilis* is not regarded as an autochthonous inhabitant of aquatic environments and does not find optimal conditions for growth, e.g. waters are poor in organic C. Therefore, proliferation is not likely to occur. Bacterial cells and especially endospores may survive, but will be subject to natural competition in the diverse micro-flora of natural waters. Survival of introduced QST 713 strain of *B. subtilis* will not cause any environmental or health impact.

B.8.3 Persistence and multiplication in air (Annex IIB 7.1.3; Annex IIIB 9)

Endospores are suitable for aerial distribution as they are easily blown about by wind (Priest, 1993, BOD2000-1370). Therefore, under conditions of use drift spacious transport may occur. Multiplication of *B. subtilis* in the air, aerosols or clouds can be excluded due to lack of organic matter supply and lack of mineral matrix to adhere to. In addition, during aerolization vegetative cells of *B. subtilis* are exposed to severe environmental stress factors (desiccation, UV-radiation, temperature), therefore survival of vegetative cells is limited (EPA, 1997, BOD2000-1366).

B.8.4 Mobility (Annex IIB 7.2; Annex IIIB 9)

An evaluation of the probable spread of *B. subtilis* into the soil or to associated environments is of minor concern, because dispersal of *B. subtilis* would lack any hazardous effects.
Estimating levels of *B. subtilis* introduced into surface waters via process wastewater discharges from facilities the U.S. EPA concluded that worst case scenarios (smaller rivers) do not suggest high levels of environmental exposure to *B. subtilis* resulting from normal fermentation operations (EPA, 1997, BOD2000-1366).
In the case of Serenade™ WP the spray application naturally enhances an aerial distribution of the active substance, QST 713 of *B. subtilis*. Considering the unfavourable conditions in the air, the overall low surface load at the site of application, and the natural distribution of *B. subtilis*, as an integral part of the soil-microflora, no detrimental concern is attributable to field applications of Serenade™ WP.

B.8.5 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[8]
AIIB-7	Anonymous	1997	Final Decision Document: TSCA Section 5(H)(4) Exemption for Bacillus subtilis. 3176 not GLP, unpublished BOD2000-1366	N	QST
AIIB-7	Asaka, O., Ano, T. and Shoda, M.	1996	Persistence of Bacillus subtilis RB14 and Its Derivative Strains in Soil with Respect to the lpa-14 Gene. not GLP, published Journal of Fermantation and Bioengineering, 81, 1, 1996, 1-6 BOD2000-1364	N	-
AIIB-7	Campbell, R.	1989	Introduction to plant pathology and microbial ecology. not GLP, published Cambridge Univertity Press, 1989, 1-40 BOD2000-1372	N	-
AIIB-7	Glick et al.	1999	Deliberate Environmental Release of Bacteria. not GLP, published Mechanisms Used by Plant-Growth Promoting Bacteria, 1999, 249-267 BOD2000-1371	N	-
AIIB-7	Phae, C.-G., Sasaki, M., Shoda, M. and Kubota, H.	1990	Characteristics of Bacillus subtilis Isolated from Composts Suppreeing Phytopthogenic Microorganisms. not GLP, published Soil Sci. Plant Nutr., 36, 4, 1990, 575-586 BOD2000-1368	N	-
AIIB-7	Priest, F.G.	1993	1. Systematics and Ecology of Bacillus. not GLP, published Journal of General Microbiology, 1993, 3-16 BOD2000-1370	N	-

[8] Only notifier listed

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[8]
AIIB-7	Siala, A. and Gray, T.R.G.	1974	Growth of Bacillus subtilis and Spore Germination in Soil Observed by a Fluorescent-antibody Technique. not GLP, published Journal of General Microbiology, 81, 1974, 191-198 BOD2000-1369	N	-
AIIB-7	van Elsas, J.D., Dijkstra, A.F., Govaert, J.M. and van Veen, J.A.	1986	Survival of Pseudomonas fluorescens and Bacillus subtilis introduced into two soils of different texture in field microplots. not GLP, published FEMS Microbiology Ecology, 38, 1986, 151-160 BOD2000-1365	N	-
AIIB-7	van Elsas, J.D., Govaert, J.M. and van Veen, J.A.	1987	Transfer of Plasmid pFT30 Between Bacilli in Soil as Influenced by Bacterial Population Dynamics and Soil Conditions. not GLP, published Soil Biol. Biochem., 19, 5, 1987, 639-647 BOD2000-1367	N	-
AIIB-2.4; AIIB-4.2; AIIB-7.1	Elsasv. J.D., Dijkstra A.F., Govaert J.M. and Veen v. J.A.	1986	Survival of Pseudomonas fluorescens and Bacillus subtilis introduced into two soils of different texture in field microplots. not GLP, published Federation of European Microbiological Societies, FEMS Microbiology Ecology 38, 1986, 151-160 BMF2000-146	N	-

Codes of owner

QST: AgraQuest, Inc.

Annex B

Bacillus subtilis strain QST 713

B-9: Ecotoxicology

B.9 Ecotoxicology

B.9.1 Effects on birds (Annex IIB 8.1; Annex IIIB 10.1)

B.9.1.1 Acute oral toxicity and/or pathogenicity and infectivity (Annex IIB 8.1)

Title:	***Bacillus subtilis*: An avian oral pathogenicity and toxicity study in the Northern bobwhite**
Author:	Foster J.W., Grimes J. and Beavers J.B.
BBA-Ref.-No.:	AVS2000-98
Test substance:	QST 713 strain of dried *Bacillus subtilis* with residual fermentation media; Lot 8AQ07D6
Activity:	2×10^{10} cfu/g
Guideline:	EPA 885.4050
Test species:	Bobwhite quail (*Colinus virginianus*)
Age:	14 d
Birds per treatment:	2 x 5 (control), 6 x 5 (treatment); sex undetermined
Exposure period:	5 days
Observation period:	25 days
Administration:	Intubation
Solvent / vehicle:	Reverse-osmosis water
Dose levels:	0/5000 mg/kg/d equivalent to $0/10^{11}$ cfu/kg/d
Findings:	Some birds of the treatment groups were observed exhibiting signs of toxicity during the exposure and post-exposure period that included depression, inability to stand, wing droop, loss of co-ordination, lethargy, reduced reaction to external stimuli and ruffled appearance. One bird was found dead on the morning of day 1. Also in the untreated control some birds exhibited minor clinical signs such as lethargy, lameness and gaping. Necropsy of the dead bird revealed non-specific findings. Necropsy of the survivors revealed no evidence of test substance replication, lesions, plaques or other evidence of pathogenicity in the treatment group. There were no effects on body weight and feed consumption.
LD_{50}:	$>10^{11}$ cfu/kg/d
valid:	yes
GLP compliance:	yes
Conclusion:	QST 713 technical has a low to moderate toxicity to birds. It is unclear which constituent of the test substance the observed effects are to be attributed to. There are no indications that the QST 713 strain of *Bacillus subtilis* is a pathogen for birds or is able to replicate in birds.

B.9.1.2 Risk assessment for birds

According to the test with bobwhite quail *Bacillus subtilis* strain QST 713 is obviously not a pathogen for birds. This is supported by the fact that it is an ubiquitous micro-organism in the environment (soil, plants, animal foodstuff) which birds naturally should come into contact with. From that reason a standard approach for the risk assessment will be conducted based on the acute exposure-toxicity-ratio.

Exposure:
The formulation Serenade WP contains 10 % *B. subtilis* strain QST 713 which is regarded here as "active substance". The application rate of 15 kg formulation/ha is equivalent to 1.5 kg as/ha. As for foliar spray in orchards residues on insects are most relevant. Using the estimate of Hoerger and Kenaga (category small insects) the residue is 1.5 * 29 = 43 mg/kg. Assuming a food intake rate for insectivorous birds of 40 % of the body weight the maximum daily intake is 17 mg/kg bw.

Toxicity:
The *Bacillus subtilis* fraction in Serenade WP has an activity of $5*10^9$ cfu/g. This is 2.5 times higher compared to the test material in the bobwhite quail test. With adjustment for this difference the LD_{50} for the "active substance" must be >2000 mg/kg.

TER
The acute TER then is >2000/17 = 117 which represents a sufficient margin of safety.

B.9.2 Effects on aquatic organisms (Annex IIB 8.2; Annex IIIB 10.2)

B.9.2.1 Acute toxicity and/or pathogenicity and infectivity to freshwater fish (Annex IIB 8.2.1)

Title:	***Bacillus subtilis*: a five-concentration toxicity and pathogenicity test with the rainbow trout (*Oncorhynchus mykiss*)**
Author:	Drottar, K.R.; Krueger, H.O. (1998)
BBA-Reference-No.:	WAT2000-897
Test substance:	QST 713 Technical (dried *Bacillus subtilis* with residual fermentation media; Lot No. 8AQ07D6; reported titer: 2×10^{10} cfu/g)
Guideline:	EPA-Pesticide Assessment Guidelines, OPPTS 885.4200 and ASTM (American Society for Testing and Materials) Standard E729-88a. (Deviations from EEC C1, Directive 92/69/EEC: exposure duration 30 d instead of 4 d, *additional* dietary exposure)
Test species:	Rainbow trout (*Oncorhynchus mykiss*)
Animals per treatment:	10 juveniles (mean weight: 2.6 g; mean length: 53 mm)

Dose levels:	0/52/86/144/240/400 mg/L, corresponding to ~1 x 10^9 - 8 x 10^9 cfu/L (renewal 3 times/week during the 30 d exposure. Additionally, fish in all treatment groups received a diet of trout chow containing 736 mg test substance/kg (~ 1.47 x 10^{10} cfu/kg))

Findings: **After 30 d of exposure rainbow trout mortality in the 144, 240 and 400 mg/L treatment groups was 30, 100 and 100 % respectively. Gross necropsy at the end of the test showed no signs of infection in gill, intestine or muscle tissue.**

LC_{50}:	162 mg/L (3.24 x 10^9 cfu/L)
NOEC:	86 mg/L (1.72 x 10^9 cfu/L)
GLP compliance:	yes (self certification by the laboratory)
Valid:	yes

B.9.2.2 Toxicity to freshwater invertebrates (Annex IIB 8.2.2)

B.9.2.2.1 Acute toxicity test with daphnia

Title:	***Bacillus subtilis*: a 48 hour static acute toxicity test with the cladoceran (*Daphnia magna*)**
Author:	Drottar, K.R.; Krueger, H.O. (1998)
BBA-Reference-No.:	WAT2000-898
Test substance:	QST 713 Technical (dried *Bacillus subtilis* with residual fermentation media; Lot No. 8AQ07D6; titer: 2 x 10^{10} cfu/g). Spray dried filtrate without *B. subtilis*, a tan powder, identified as 32-45-14B, lot # 812-0919.
Guideline:	EPA Series 72 of Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms; EPA 540/9-82-024 EPA Standard Evaluation Procedure, Acute Toxicity Test for Freshwater Invertebrates. Hazard Evaluation Division. Office of Pesticide Programs. EPA 540/9-85-005 ASTM (American Society for Testing and Materials) Standard E 729-88a (1994) Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians.
Test species:	*Daphnia magna*
Animals per treatment:	2 x 10 neonates per control group and per concentration in treatment group
Dose levels:	0/13/25/50/100/200 mg/L (One control group was exposed to a spray dried filtrate of fermentation material without *B. subtilis)*
Findings:	Daphnids in the control group exposed to the spray dried filtrate without *B. subtilis* appeared as normal and healthy as daphnids in the negative control group. Mortality in the 25, 50, 100 and 200 mg/L treatment groups was 15, 15, 45 and 85 %, respectively.

EC_{50} (24 h):	147 mg/L (calculated from mortality/immobility data)
EC_{50} (48 h):	108 mg/L
NOEC:	13 mg/L
GLP compliance:	yes (self certification by the laboratory)
Valid:	yes

B.9.2.2.2 Chronic (21-day) toxicity test with daphnia

Title:	***Bacillus subtilis*: a 21-day life cycle toxicity and pathenogenicity test with the cladoceran (*Daphnia magna*)**
Author:	Drottar, K.R.; Krueger, H.O. (1998)
BBA-Reference-No.:	WAT2000-899
Test substance:	QST 713 Technical (dried *Bacillus subtilis* with residual fermentation media; Lot No. 8AQ07D6; titer: 2 x 10^{10} cfu/g).
Guideline:	EPA Microbial Pesticide Test Guidelines OPPTS 885.4240 ASTM Standard E 1193-87 Standard Guide for Conducting Renewal Life-Cycle Toxicity Tests with *Daphnia magna*. Deviations: Slightly lower test concentration applied compared to OPPTS guideline (recommended minimum concentration in test water: 1 x 10^6 cfu/mL). Justification: selection of test concentrations were based upon the results of a range-finding toxicity test.
Test species:	*Daphnia magna*
Animals per treatment:	4 x 5 neonates per control group and per concentration in treatment group
Dose levels:	0/1.9/3.8/7.5/15.0/30.0 mg/L (corresponding to 3.8 x 10^7, 7.6 x 10^7, 1.5 x 10^8, 3.0 x 10^8 and 6.0 x 10^8 cfu/L respectively), renewal 3 times/ week.
Findings:	None of the tested concentrations caused ≥ 50% mortality or immobility. Test substance concentrations up to 7.5 mg/L did not cause significant reduction in survival, reproduction or growth. *Daphnia magna* exposed to 15 mg/L showed significant reduction in reproduction, mean length and dry weight. The **MATC** (*m*aximum *a*cceptable *t*oxicant *c*oncentration) was calculated to be 10.6 mg/L (= 2.1 x 10^8 cfu/L) - as the geometric mean of the NOEC and the LOEC.
EC_{50} (21 d):	> 30 mg/L (mortality or immobility)
NOEC:	7.5 mg/L (= 1,5 x 10^8 cfu/L)
LOEC:	15.0 mg/L (= 3.0 x 10^8 cfu/L)
GLP compliance:	yes (self certification by the laboratory)
Valid:	yes

B.9.2.3 Effects on algae growth (Annex IIB, 8.2.3)

Title:	**Testing of toxic effects of QST 713 TP on the single cell green alga *Scenedesmus subspicatus***
Author:	Dengler, D. (2000)
BBA-Reference-No.:	WAT2000-900
Test substance:	QST 713 Technical (dried *Bacillus subtilis* with residual fermentation media; Lot No. 8AQ07F1 / Drum 20, reported titer: 3.3×10^9 cfu/g)
Guideline:	OECD 201. Deviations: The range-finding test was performed in a limit-test design (Control and 100 mg/L with 6 replicates each, other concentrations with 1 replicate each). As there was no evidence of inhibitory effects of QST 713 TP at any test concentration, the results of the range-finding test were used for evaluation.
Test species:	*Scenedesmus subspicatus*
Animals per treatment:	Not applicable
Dose levels:	0.01, 0.1, 1, 10 and 100 mg/L of test substance.
Findings:	Concentrations of test substance (cfu in test solutions) maintained sufficiently stable during the test. No adverse effects of QST 713 TP were observed at any test concentration. Therefore, no EC values could be calculated. A stimulation of the growth rate of ca. 18 % was observed at test substance concentrations of 1 and 10 mg/L. However a statement on the significance of this stimulation is not possible due to the limited number of replicates.
E_bC_{50}:	not determined
E_rC_{50}:	not determined
NOEC:	not determined (100 mg/L)
LOEC:	not determined (≥ 100 mg/L)
GLP compliance:	yes (self certification by the laboratory)
Valid:	yes

B.9.2.4 Exposure and risk assessment for aquatic organisms

From the results of the studies described above it can be concluded that QST 713 TP is of low toxicity to aquatic organisms. According to the Draft Guidance Document on Aquatic Ecotoxicology (8075/VI/97 rev. 7, 08.07.2000) the predicted initial environmental concentration in surface water can be calculated taking into account the drift rates reported by Ganzelmeier et al. (1995). When applied during early growth stages in fruit crops (worst case), spray drift amounts to 29.6 % in a distance of 3 m from application. On the basis of the maximum application rate of 15 kg/ha (i.e. 1.5 kg as/ha), an initial PEC_{sw} of 0.148 mg as/L in the top 30 cm water layer of lentic water bodies can be calculated.

Table B.9.2-1 summarises TER-values calculated for a single application with the maximum application rate of 15 kg/ha (i.e. 1.5 kg as/ha).

Table B.9.2-1: QST 713 TP – Toxicity exposure ratios (TER) for aquatic organisms (single application of 1.5 kg as/ha; 29.6 % spray drift in a distance of 3 m; early growth stage in fruit crops)

Test species	Toxicity [mg as/L]	Initial PEC_{sw} [mg as/L]	TER	Annex VI Trigger
O. mykiss	162	0.148	1095	100
D. magna	108	0.148	723	100
S. subspicatus	≥ 100	0.148	≥ 676	10

All acute TER-values are well above the relevant Annex VI trigger. Therefore the risk to aquatic organisms arising from acute exposure is considered to be acceptable. The applicant also submitted a chronic (21 d) toxicity test with *Daphnia magna*. A chronic TER of 51 can be derived by comparing the NOEC of 7.5 mg/L with the initial PEC_{sw} of 0.148 mg as/L, assuming worst case conditions as described above. This value is well above the relevant Annex VI trigger.

All toxicity values exceed the limit values for toxic or adverse effects. Therefore a hazard classification or specific labelling according to Directive 67/548/EEC is not necessary.

In conclusion, the overall risk to aquatic organisms is considered to be acceptable.

B.9.3 Effects on bees (Annex IIB 8.3; Annex IIIB 10.3)

B.9.3.1 Oral toxicity of *Bacillus subtilis*-strain QST 713 to honeybees

Report:
Hoxter, Kimberly A; Palmer, Susanne J.; Krueger, Henry O. (1998, BIE2000-27): *Bacillus subtilis*: a dietary pathogenicity and toxicity study with the honey bee (*Apis mellifera*); Wildlife International Ltd., Easten, Maryland, U.S.A., unpublished; Projekt No.: 489-102 C; dates of experimental work: June, 1998 - Aug. 27.1998.

Guidelines:
EPA Microbial Pesticide Test Guidelines OPPTS 885.4380

Corresponds generally to OECD guideline 213 (applying to chemical substances)

Deviations: Observation period 5 days instead of recommended 3 days after exposure.
No toxic standard is used.

GLP:
Yes (self certification by the laboratory).

Material and methods:
Test substance: QST 713 Technical (dried *Bacillus subtilis* with residual fermentation media; Lot No. 8AQ7D6; reported titer: 2 x 10^{10} cfu/g)
5 day feeding test: the test substance was administered in a honey/water diet ad libitum to honeybees (6 replicated test chambers x 20 bees per control group and per concentration in treatment group) for a period of 5 days. Applied test substance concentrations in the diet were 600, 6000 and 60 000 ppm (relating to factor 1, 10 and 100 of EEC (Estimated Environmental Concentration) - equivalent to 1.2 x 10^7 – 1.2 x 10^8 and 1,2 x 10^9 cfu/ml of diet.
Observations were made twice within the first 4 h of test initiation and then daily until negative control mortality exceeded 20 % on Day 5 of the test. Mortality in treatment groups was adjusted for control mortality.

Findings:
Clinical signs as immobility, lethargy or loss of equilibrium were exhibited by a few bees in all treatment groups (starting on Day 0 in the higher dosed groups and on Day 3 in the lowest concentration group).
Treatment related mortality was dose responsive. Considerable increase in mortality occurred by Day 2 in the highest dosage group (receiving 60 000 ppm).
Dietary LC_{50}: ~8900 ppm (equivalent to 1.8 x 10^8 cfu/ml diet) – corresponding to approximately 15 times the reported ECC (Estimated Environmental Concentration) derived from the maximum application rate (= 10 lb/a = 11.22 kg/ha).

B.9.3.2 Bee brood feeding test

No data submitted

B.9.3.3 Residue test

No data submitted

B.9.3.4 Cage test

No data submitted

B.9.3.5 Field test

No data submitted

B.9.3.6 Tunnel test

No data submitted

B.9.3.7 Risk assessment for bees

Basically the calculation of a hazard quotient according to the EPPO/CoE risk assessment scheme for chemicals is not applicable to micro organism, as the ratio of a maximum application rate of as to a LD_{50} value would not represent the potential risk of a micro organism for the test organism, since an infective organism would be able to reproduce and colonize the potential host, regardless of the initially consumed amount. But the high concentration of *Bacillus subtilis* (Dietary LC_{50}: ~ 8900 ppm, equivalent to ~ 1.8×10^8 cfu/ml diet) consumed ad libitum over a testing period of 5 days does not indicate a risk for bees by the practical use of *Bacillus subtilis* products.

B.9.4 Effects on non-target arthropods other than bees (Annex IIB 8.4; Annex IIIB 10.4)

The results presented below are considered valid (i.e. quality criteria are fulfilled). The risk assessment is based on the uses and nominal field rates outlined in this monograph. Investigations into the acute toxicity and pathogenecity and infectivity of *Bacillus subtilis* are conducted using a representative formulation as suggested in the SETAC/ESCORT "*Guidance document on regulatory testing procedures for pesticides with non-target arthropods*" (Barrett et al., 1994).

B.9.4.1 Acute toxicity, pathogenecity and infectivity (Annex IIB 8.5, Annex IIIB 10.2.2)

Investigations into the acute toxicity and pathogenecity and infectivity of formulated *Bacillus subtilis* in laboratory tests:

Predatory mites

Title:	**QST 713 TP: Toxicity to the Predatory Mite, *Typhlodromus pyri Scheuten* (Acari, Phytoseiidae) in the Laboratory.**
Author:	Adelberger, I. (2000)
BBA-Ref.-No.:	ANA2000-898
Test substance:	Formulation Bacillus subtilis (Serenade WP) *Bacillus subtilis*
Guideline:	Typhlodromus (Louis und Ufer 1995)
Test species:	*Typhlodromus pyri*
Developmental stage:	Protonymphs
Substrate:	Glass plates
Exposure route:	deposit
Exposure duration:	14 d (7 + 7)

Results:

Appl. rate	Mortality	Sublethal effects
16 kg/ha	30.7 %	13.04 % (Fertility)

Remarks: "Serenade WP" contains 10^8 CFU/g (nominal), $\geq$ 10^6 CFU/g *Bacillus subtilis* analysed.
valid: yes
GLP compliance: yes

Parasitoids

Title: **A dietary pathogenicity and toxicity study with the Parasitic Hymenoptera (*Nasonia vitripennis*).**
Author: Hoxter, K., Palmer, S., Krueger, H. (1998)
BBA-Ref.-No.: ANA2000-896
Test substance: Formulation Bacillus subtilis (Serenade WP)
Bacillus subtilis
Guideline: US EPA, Series 885, OPPTS Number 885.4340
Test species: *Nasonia vitripennis*
Developmental stage: Adults
Substrate: cotton swab coated with diet
Exposure route: oral
Exposure duration: 15 d
Results:

Appl. rate	Mortality	Sublethal effects
0.9 kg/ha	46.15 %	none
9 kg/ha	19.23 %	negligible
90 kg/ha	65.39 %	negligible

Remarks: Reported activity of test substance: $2{,}0 \cdot 10^{10}$ CFU/g. Oral administration: 0,03 g/50 ml diet (equivalent 0.9 kg/ha in 1500 l water); 0,3 g/50 ml (equivalent 9 kg/ha in 1500 l water); 3 g/50 ml (equivalent 90 kg/ha in 1500 l water).
valid: yes
GLP compliance: yes

Title: **QST 713 TP: Acute Toxicity to the Aphid Parasitoid, *Aphidius rhopalosiphi* (Hymenoptera, Braconidae) DeStefani-Perez in the Laboratory.**
Author: Schuld, M. (2000)
BBA-Ref.-No.: ANA2000-897
Test substance: Formulation Bacillus subtilis (Serenade)
Bacillus subtilis
Guideline: Aphidius, Lab (Mead-Briggs 1992)
Test species: *Aphidius rhopalosiphi*
Developmental stage: Imagines
Substrate: Glass plates

Exposure route: deposit
Exposure duration: 48 h
Results:

Appl. rate	Mortality	Sublethal effects
16 kg/ha	5.13 %	25.3 % (Parasitation capacity)

Remarks: "Serenade WP" contains 10^8 CFU/g (nominal), $\geq 10^6$ CFU/g *Bacillus subtilis* analysed.
valid: yes
GLP compliance: yes

Plant dwelling species

Title: **A dietary pathogenicity and toxicity study with the Green Lacewing Larvae (*Chrysoperla carnea*).**
Author: Hoxter, K., Palmer, S., Krueger, H. (1998)
BBA-Ref.-No.: ANA2000-895
Test substance: Formulation Bacillus subtilis (Serenade WP)
Bacillus subtilis
Guideline: US EPA, Series 885, OPPTS Number 885.4340
Test species: *Chrysopa carnea*
Developmental stage: Larvae
Substrate: moist, grainy meal from moth eggs treated with test solution
Exposure route: oral
Exposure duration: 13 d
Results:

Appl. rate	Mortality	Sublethal effects
0.9 kg/ha	0.5 %	none
9 kg/ha	47.6 %	none
90 kg/ha	26.3 %	none

Remarks: Mortality covers larval mortality and pupation rate. Reported activity of test substance: $2{,}0 \cdot 10^{10}$ CFU/g. Oral administration: 0,18 g/50 ml test substance stock, 1 g of stock in 6 g of diet (equivalent 0.9 kg/ha in 1500 l water); 1,8 g/50 ml test substance stock, 1 g of stock in 6 g of diet (equivalent 9 kg/ha in 1500 l water); 18 g/50 ml test substance stock, 1 g of stock in 6 g of diet (equivalent 90 kg/ha in 1500 l water).
valid: yes
GLP compliance: yes

Title: **A dietary pathogenicity and toxicity study with the Ladybird Beetle (*Hippodamia convergens*).**
Author: Hoxter, K., Palmer, S., Krueger, H. (1998)
BBA-Ref.-No.: ANA2000-894
Test substance: Formulation Bacillus subtilis (Serenade WP)
Bacillus subtilis
Guideline: US EPA, Series 885, OPPTS Number 885.4340
Test species: *Hippodamia convergens*

Developmental stage: Adults
Substrate: cotton swab coated with diet from honey, special ladybird beetle diet and test substance
Exposure route: oral
Exposure duration: 30 d
Results:

Appl. rate	Mortality	Sublethal effects
0.9 kg/ha	11.8 %	negligible
9 kg/ha	4.7 %	negligible
90 kg/ha	2.4 %	negligible

Remarks: Reported activity of test substance: $2{,}0 \cdot 10^{10}$ CFU/g. Oral administration: 0,03 g/50 ml diet (equivalent 0.9 kg/ha in 1500 l water); 0,3 g/50 ml (equivalent 9 kg/ha in 1500 l water); 3 g/50 ml (equivalent 90 kg/ha in 1500 l water).
valid: yes
GLP compliance: yes

Table B.9.4-1: Summary of arthropod toxicity data with the formulation Serenade WP

Test material	Species	Develop-mental stage	Sub-strate	Dosage kg/ha	Effects %	
					lethal	sublethal
Predatory mites						
Bacillus subtilis (Serenade WP)	*T. pyri*	Protonymphs	I	16	30.7	13. 04
Parasitoids						
Bacillus subtilis (Serenade)	*A. rhopalosiphi*	Adults	I	16	5.13	25.3
Bacillus subtilis (Serenade WP)	*N. vitripennis*	Adults	I	0.9	46.15	
Bacillus subtilis (Serenade WP)	*N. vitripennis*	Adults	I	9	19.23	
Bacillus subtilis (Serenade WP)	*N. vitripennis*	Adults	I	90	65.39	
Plant dwelling species						
Bacillus subtilis (Serenade WP)	*C. carnea*	Larvae	I	0.9	0.5	
Bacillus subtilis (Serenade WP)	*C. carnea*	Larvae	I	9	47.6	
Bacillus subtilis (Serenade WP)	*C. carnea*	Larvae	I	90	26.3	
Bacillus subtilis (Serenade WP)	*H. convergens*	Adults	I	0.9	11.8	
Bacillus subtilis (Serenade WP)	*H. convergens*	Adults	I	9	4.7	
Bacillus subtilis (Serenade WP)	*H. convergens*	Adults	I	90	2.4	

I = Inert substrate,

B.9.4.2 Field tests (Annex IIIB 10.2.2)

Field and semi-field tests

not required

B.9.4.3 Risk assessment for non-target terrestrial arthropods

Intended uses of plant protection products containing *Bacillus subtilis* cover max. 16 applications per season of max. 5 to 15 kg formulated product · ha^{-1} to control fungal diseases in fruits, 8 applications of max 4 to 12 kg formulated product · ha^{-1} in grapes and and lettuce. Non-target arthropods are likely to be exposed to formulated *Bacillus subtilis* by direct spray, contact on fresh or dry residues, oral uptake of contaminated pollen, nectar and honey dew and prey. Contact via host organisms is considered of minor importance. As a tier 1 worst-case exposure scenario, the predicted initial environmental exposure of non-target arthropods is assumed to be equivalent to the maximum nominal field rate of 15 kg · ha^{-1} times MAF of 3.5 (multiple application factor, ECPA, 2000, in press), making 52.5 kg · ha^{-1}.

The field rates tested given in Table B.9.4-1: Summary of arthropod toxicity data with the formulation Serenade WP compare to the nominal single intended field rates, some covering twice the max. estimated exposure caused by multiple applications as calculated according to ECPA. According to the data submitted some rather low inherent toxicity was demonstrated in basic laboratory tests. No signs of pathogenecity and infectivity of formulated *Bacillus subtilis* were reported. Sublethal effects found were jugded negligible, because only single individuals were affected. However, no dose-response-relationship was apparent. The effects reported are not considered unacceptable.

It is therefore established in the light of current scientific and technical knowledge and as laid down in the SETAC/ESCORT *"Guidance document on regulatory testing procedures for pesticides with non-target arthropods*" (Barrett et al., 1994), that the use of *Bacillus subtilis* as outlined in this monograph has no unacceptable influence on non-target arthropods, represented by species of three ecological groups.

B.9.5 Effects on earthworms (Annex IIB 8.5; Annex IIIB 10.5)

No data have been submitted.

Bacillus subtilis is an autochthonous micro-organism of the soil. However, effects of strain QST 713 on earthworms can not be excluded, as there is no information on the natural occurrence of this specific strain in soil. Application of the formulated product also might enhance the potential exposure concentration for earthworms. In addition, there are indications from a study on mice where two out of four tested strains exhibited toxicity after intraperitoneal injection.

The possible risk of *Bacillus subtilis* strain QST 713 should be addressed by conducting an acute study combined with a histopathological investigation of the earthworm tissue.

B.9.6 Additional studies (Annex IIB 8.6; Annex IIIB 10.6)

B. subtilis is a viable micro-organism of ubiquitous occurrence. The strain QST 713 has originally been isolated from soil in California, U.S.A., therefore its possible multiplication in this natural habitat does not affect the natural micro-flora.
The phenomenon of fast decreasing vegetative cell numbers is reported for a *B. subtilis* strain introduced into soil (Asaka et al. 1996), while in parallel sporulation increased. After a few days the cell population was shown to be stabilised as endospores.
As vegetative growth declines as the nutrient source declines this species does not seem to compete well for limited resources and *B. subtilis* populations will be subject to competition in the natural micro-flora (Campbell 1989, BMF2000-149). With regard to the intended fields of use (vineyards, orchards and lettuce fields) most organic matter will be supplied to the soil in orchards in spring, with the end of flowering, and in autumns, at leaf fall. Only in springtime Serenade™ WP applications will coincide with significantly increased organic matter supply to the soil surface.
Finally, introduced *B. subtilis* cells are not expected to exceed the natural level permanently.
Therefore no additional studies are necessary.

B.9.7 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[9]
AIIA-8.1	Foster, J.W.; Grimes, J. and Beavers, J.B.	1998	Bacillus subtilis: An avian oral pathogenicity and toxicity study in the northern bobwhite. 489-101 GLP, unpublished AVS2000-98	Y	QST
AIIA-8.3.2	Adelberger, I.	2000	QST 713 TP: Toxicity to the Predatory mite, Typhlodromus pyri Scheuten (Acari, Phytoseiidae) in the Laboratory. 99431/01-NLTp GLP, unpublished ANA2000-898	Y	QST
AIIA-8.3.2	Hoxter, K., Palmer, S., Krueger, H.	1998	A dietary pathogenicity and toxicity study with the Parasitic Hymenoptera (Nasonia vitripennis). 489-105A GLP, unpublished ANA2000-896	Y	QST

[9] Only notifier listed

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[9]
AIIA-8.3.2	Hoxter, K., Palmer, S., Krueger, H.	1998	A dietary pathogenicity and toxicity study with the Green Lacewing Larvae (Chrysoperla carnea). 489-104 GLP, unpublished ANA2000-895	Y	QST
AIIA-8.3.2	Hoxter, K., Palmer, S., Krueger, H.	1998	A dietary pathogenicity and toxicity study with the Ladydird Beetle (Hippodamia convergens). 489-103B GLP, unpublished ANA2000-894	Y	QST
AIIA-8.3.2	Schuld, M.	2000	QST 713 TP: Acute Toxicity to the Aphid Parasitoid, Aphidius rhopalosiphi (Hymenoptera, Braconidae) DeStefani-Perez in the Laboratory. 99431/01-NLAp GLP, unpublished ANA2000-897	Y	QST
AIIB-8.2	Dengler, D.	2000	Testing of toxic effects of QST 713 TP on the single cell green alga Scenedesmus subspicatus. 99431/01-AASs GLP, unpublished WAT2000-900	Y	QST
AIIB-8.2	Drottar, K.R., Krueger, H.O.	1998	Bacillus subtilis: A 21-day life-cycle toxicity and pathogenicity test with the cladoceran (Daphnia magna). 489A-102B GLP, unpublished WAT2000-899	Y	QST
AIIB-8.2	Drottar, K.R., Krueger, H.O.	1998	Bacillus subtilis: A 48-hour static acute toxicity test with the cladoceran (Daphnia magna). 489A-103 GLP, unpublished WAT2000-898	Y	QST

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[9]
AIIB-8.2	Drottar, K.R., Krueger, H.O.	1998	Bacillus subtilis: A five-concentration toxicity and pathogenicity test with the rainbow trout (Oncorhynchus mykiss). 489A-101 GLP, unpublished WAT2000-897	Y	QST
AIIB-8.2	Thobor, C.	2000	Bacillus subtilis (150 g/kg technical powder) & Serenade TM WP (ai: 100 g/kg wettable powder) Summary of toxicity, pathogenicity and infectivity to non-target organisms, risk assessment and overall evaluations. AB981202-EU09/02 not GLP, unpublished WAT2000-896	N	QST
AIIIA-10.4	Hoxter, Kimberly A.;Palmer, Susanne J.; Krueger, Henry O.	1998	Bacillus subtilis: A Dietary pathogenicity and toxicity study with the honey bee (Apis mellifera). 489-102C GLP, unpublished BIE2000-27	Y	QST
AIIIA-10.6.1	Asaka, O.; Ano, T.; Shoda, M.	1996	Persistence of Bacillus subtilis RB14 and its derivative strain in soil with respect to the lpa-14 Gene. not GLP, published J. of Fementation and Bioengineering, 81, 1, 1996, 1-6 ARW2000-129	N	-
AIIIA-10.6.1	Barrett et al.	1994	Guidance document on regulatory testing procedures for pesticides and non-target arthropods. Escord Workshop held at Wageningen 28-30 march 1994. not GLP, published , 1994 ARW2000-130	N	-
AIIIA-10.6.1	Elsas v. J.D.; Dijkstra, A.F.; Govaert, J.M.; Veen v., J.A.	1986	Survival of pseudomonas fluorescens and bacillus subtilis introduced into two soils of different texture in field microplots. not GLP, published FEMS Microbiology Ecology, 38, 1986, 151-160 ARW2000-131	N	-

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[9]
AIIIA-10.6.1	Elsas v., J.D.; Govaert, J.M. ; Veen v., J.A.	1987	Transfer of plasmid pFT30 between bacilli in soil as influenced by bacterial population dynamics and soil conditions. not GLP, published Soil Biol. Biochem., 19, 5, 1987, 639-647 ARW2000-132	N	-
AIIIA-10.6.1	EPA		Final decision document, TSCA section 5 (H) (4) exemption for Bacillus subtilis + Attachment 1. not GLP, published ARW2000-134	N	-
AIIIA-10.6.1	Phae, C.-G., Sasaki, M.; Shoda, M.; Kubota, H.	1990	Characteristics of Bacillus subtilis isolated from composts suppressing phytopatho-genic microorganisms. not GLP, published Soil Sci Plant Nutr., 36, 4, 1990, 575-586 ARW2000-136	N	-
AIIIA-10.6.1	Siala, A. Gray, T.R.G.	1974	Growth of bacillus subtilis and spore germination in soil observed by a fluorescent-antibody technique. not GLP, published J. of General Microbiology, 81, 1974, 191-198 ARW2000-137	N	-

Codes of owner

QST: AgraQuest, Inc.

Appendix 1

Bacillus subtilis strain QST 713

Standard Terms and Abbreviations

B.10 Appendices

B.10.1 Appendix I: Standard terms and abbreviations

Part 1 Technical Terms

A	ampere
ACH	acetylcholine
AChE	acetylcholinesterase
ADI	acceptable daily intake
ADP	adenosin diphosphate
AE	acid equivalent
AFID	alkali flame-ionization detector or detection
A/G	albumin/globulin ratio
ai	active ingredient
ALD_{50}	approximate median lethal dose, 50 %
ALT	alanine amitrotransferase (SGPT)
AMD	automatic multiple development
ANOVA	analysis of variance
AOEL	acceptable operator exposure level
AP	alkaline phosphatase
approx	approximate
ARC	anticipated residue contribution
ARfD	acute reference dose
as	active substance
AST	aspartate aminotransferase (SGOT)
ASV	air saturation value
ATP	adenosine triphosphate
BCF	bioconcentration factor
bfa	body fluid assay
BOD	biological oxygen demand
bp	boiling point
BSAF	biota-sediment accumulation factor
BSE	bovine spongiform encephalopathie
BSP	bromosulfophthalein
Bt	bacillus thuringiensis
Bti	bacillus thuringiensis israelensis
Btk	bacillus thuringiensis kurstaki
Btt	bacillus thuringiensis tenebrionis
BUN	blood urea nitrogen
bw	body weight
c	centi- (x 10^{-2})
°C	degree celsius (centigrade)
CA	controlled atmosphere
CAD	computer aided design
CADDY	computer aided dossier and data supply (an electronic dossier interchange and archiving format)
cd	candela
CDA	controlled drop(let) application

cDNA	complementary DNA
CEC	cation exchange capacity
cf	confer, compare to
CFU	colony forming units
ChE	cholinesterase
CI	confidence interval
CL	confidence limits
cm	centimetre
CNS	central nervous system
COD	chemical oxygen demand
CPK	creatinine phosphatase
cv	coeficient of variation
Cv	ceiling value
CXL	Codex Maximum Residue Limit (Codex MRL)
d	day
DES	diethylstilboestrol
DFR	dislodgeable foliar residue
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
dna	designated national authority
DO	dissolved oxygen
DOC	dissolved organic carbon
dpi	days pot inoculation
DRES	dietary risk evaluation system
DT_{50}	period required for 50 percent dissipation (define method of estimation)
DT_{90}	period required for 90 percent dissipation (define method of estimation)
dw	dry weight
DWQG	drinking water quality guidelines
ε	decadic molar extinction coefficient
EC_{50}	effective concentration
ECD	electron capture detector
ECU	European currency unit
ED_{50}	median effective dose
EDI	estimated daily intake
ELISA	enzyme linked immunosorbent assay
e-mail	electronic mail
EMDI	estimated maximum daily intake
EPMA	electron probe micro analysis
ERC	environmentally relevant concentration
ERL	extraneous residue limit
F	field
F_0	parental generation
F_1	filial generation, first
F_2	filial generation, second
FIA	fluorescence immuno assay
FID	flame ionization detector
FOB	functional observation battery
fp	freezing point
FPD	flame photometric detector

FPLC	fast protein liquid chromatography
g	gram
G	glasshouse
GAP	good agricultural practice
GC	gas chromatography
GC-EC	gas chromatography with electron capture detector
GC-FID	gas chromatography with flame ionization detector
GC-MS	gas chromatography-mass spectrometry
GC-MSD	gas chromatography with mass-selective detection
GEP	good experimental practice
GFP	good field practice
GGT	gamma glutamyl transferase
GI	gastro-intestinal
GIT	gastro-intestinal tract
GL	guideline level
GLC	gas liquid chromatography
GLP	good laboratory practice
GM	geometric mean
GMO	genetically modified organism
GMM	genetically modified micro-organism
GPC	gel-permeation chromatography
GPPP	good plant protection practice
GPS	global positioning system
GSH	glutathion
GV	granulosevirus
h	hour(s)
H	Henry`s Law constant (calculated as a unitless value) (see also K)
ha	hectare
Hb	haemoglobin
HCG	human chorionic gonadotropin
Hct	haematocrit
HDT	highest dose tested
hL	hectolitre
HEED	high energy electron diffraction
HID	helium ionization detector
HPAEC	high performance anion exchange chromatography
HPLC	high pressure liquid chromatography or high performance liquid chromatography
HPLC-MS	high pressure liquid chromatography – mass spectrometry
HPPLC	high pressure planar liquid chromatography
HPTLC	high performance thin layer chromatography
HRGC	high resolution gaschromatography
Hs	Shannon-Weaver index
Ht	haematocrit
I	indoor
I_{50}	inhibitory dose, 50 %
IC_{50}	median immobilization concentration
ICM	integrated crop management
ID	ionization detector

IEDI	international estimated daily intake
IGR	insect growth regulator
im	intramuscular
inh	inhalation
ip	intraperitoneal
IPM	integrated pest management
IR	infrared
ISBN	international standard book number
ISSN	international standard serial number
iv	intravenous
IVF	in vitro fertilization
k	kilo
K	Kelvin or Henry`s Law constant (in athmospheres per cubic meter per mole) (see also H)13
K_{ads}	adsorption constant
K_{des}	apparent desorption coefficient
K_{oc}	organic carbon adsortion coefficient
K_{om}	organis matter adsorption coefficient
kg	kilogram
L	litre
LAN	local area network
LASER	light amplification by stimulated emission
LBC	loosely bound capacity
LC	liquid chromatography
LC-MS	liquid chromatography-mass spectrometry
LC_{50}	lethal concentration, median
LCA	life cycle analysis
LCLo	lethal concentration low
LC-MS-MS	liquid chromatography with tandem mass spectrometry
LD_{50}	lethal dose, median; dosis letalis media
LDLo	lethal dose low
LDH	lactate dehydrogenase
LOAEC	lowest observable adverse effect concentration
LOAEL	lowest observable adverse effect level
LOD	limit of determination
LOEC	lowest observable effect concentration
LOEL	lowest observable effect level
LOQ	limit of quantification (determination)
LPLC	low pressure liquid chromatography
LSC	liquid scintillation counting or counter
LSD	least squared denominator multiple range test
LSS	liquid scintillation spectrometry
LT	lethal threshold
m	metre
M	molar
µm	micrometer (micron)
MC	moisture content
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentratin

MCV	mean corpuscular volume
MDL	method detection limit
MFO	mixed function oxidase
µg	microgram
mg	milligram
MHC	moisture holding capacity
min	minute(s)
mL	millilitre
MLT	median lethal time
MLD	minimum lethal dose
mm	millimetre
mo	month(s)
mol	Mol
MOS	margin of safety
mp	melting point
MRE	maximum residue expected
MRL	maximum residue limit or level
mRNA	messenger ribonucleic acid
MS	mass spectrometry
MSDS	material safety data sheet
MTD	maximum tolerated dose
n	normal (defining isomeric configuration)
NAEL	no adverse effect level
nd	not detected
NEDI	no effect daily intake (mg/kg body wt/day)
NEL	no effect level
NERL	no effect residue level
ng	nanogram
nm	nanometer
NMR	nuclear magnetic resonance
no	number
NOAEC	no observed adverse effect concentration
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
NOED	no observed effect dose
NOEL	no observed effect level
NOIS	notice of intend to suspend
NPD	nitrogen-phosphorus detector or detection
NPV	nuclear polyhedrosis virus
NR	not reported
NTE	neurotoxic target esterase
OC	organic carbon content
OCR	optical character recognition
ODP	ozone-depleting potential
ODS	ozone-depleting substances
OM	organic matter content
op	organophosphorus pesticide
Pa	pascal
PAD	pulsed amperometric detection

2-PAM	2-pralidoxime
pc	paper chromatography
PC	personal computer
PCV	haematocrit (packed corpuscular volume)
PEC	predicted environmental concentration
PEC_A	predicted environmental concentration in air
PEC_S	predicted environmental concentration in soil
PEC_{SW}	predicted environmental concentration in surface water
PEC_{GW}	predicted environmental concentration in ground water
PED	plasma-emissions-detektor
pH	pH-value
PHED	pesticide handler`s exposure data
PHI	pre-harvest interval
PIC	prior informed consent
pic	phage inhibition capacity
PIXE	proton inducted X-ray emission
pK_a	negative logarithm (to the base 10) of the dissociation constant)
PNEC	predicted no effect concentration
po	by mouth (per os)
P_{ow}	partition coefficient between n-octanol and water
POP	persistent organic pollutants
ppb	parts per billion (10^{-9})
PPE	personal protective equipment
ppm	parts per million (10^{-6})
ppp	plant protection product
ppq	parts per quadrillion (10-24)
ppt	parts per trillion (10^{-12})
PSP	phenolsulfophthalein
PrT	prothrombin time
PRL	practical residue limit
PT	prothrombin time
PTDI	provisional tolerable daily intake
PTT	partial thromboplastin time
QSAR	quantitative structure-activity relationship
r	correlation coefficient
r^2	coefficient of determination
RBC	red blood cell
REI	restricted entry interval
R_f	ratio of fronts
RfD	reference dose
RH	relative humidity
RL_{50}	residual lifetime
RNA	ribonucleic acid
RP	reversed phase
rpm	reversed phase material
rRNA	ribosomal ribonucleic acic
RRT	relative retention time
RSD	relative standard deviation
s	second

SAC	strong adsorption capacity
SAP	serum alkaline phosphatase
SAR	structure/activity relationship
SBLC	shallow bed liquid chromatography
sc	subcutaneous
sce	sister chromatid exchange
SD	standard deviation
SE	standard error
SEM	standard error of the mean
SEP	standard evaluation procedure
SF	safety factor
SFC	supercritical fluid chromatography
SFE	supercritical fluid extraction
SIMS	secondary ion mass spectroscopy
SOP	standard operating procedures
sp	species (only after a generic name)
SPE	solid phase extraction
SPF	specific pathogene free
spp	subspecies
sq	square
SSD	sulfur specific detector
SSMS	spark source mass spectrometry
STEL	short term exposure limit
STMR	supervised trials median residue
t	tonne (metric ton)
$t_{1/2}$	half-life (define method of estimination)
T_3	tri-iodothyroxine
T_4	thyroxine
TADI	temporary acceptable daily tntake
TBC	tightly bound capacity
TCD	thermal conductivity detector
TCLo	toxic concentration low
TID	thermionic detector, alkali flame detector
TDLo	toxic dose low
TDR	time domain reflectrometry
TER	toxicity exposure ration
TER_I	toxicity exposure ration for initial exposure
TER_{ST}	toxicity exposure ration following repeated exposure
TER_{LT}	toxicity exposure ration following chronic exposure
tert	tertiary (in a chemical name)
TEP	typical end-use product
TGGE	temperature gradient gel electrophoresis
TIFF	tag image file format
TLC	thin layer chromatography
Tlm	median tolerance limit
TLV	threshold limit value
TMDI	theoretical maximum daily intake
TMRC	theoretical maximum residue contribution
TMRL	temporary maximum residue limit

TOC	total organic chlorine
Tremcard	Transport emergency card
tRNA	transfer riboncleic acid
TSH	thyroid stimulating hormone (thyrotropin)
TWA	time weighted average
UDS	unscheduled DNA synthesis
UF	uncertainly factor (safety factor)
ULV	ultra low volume
UV	ultraviolet
v/v	volume ratio (volume per volume)
WBC	white blood cell
wk	week
wt	weight
w/v	weight per volume
w/w	weight per weight
XRFA	X-ray fluorescence analysis
yr	year
<	less than
≤	less than or equal to
>	greater than
≥	greater than or equal to

Part 2 Organisations and Publications

ACPA	American Crop Protection Association
ACTM	American Society for Testing and Materials
BA	Biological Abstracts (Philadelphia)
BART	Beneficial Arthropod Registration Testing Group
CA	Chemical Abstracts
CAB	Centre for Agriculture and Biosciences International
CAC	Codex Alimentarius Commission
CAS	Chemical Abstracts Service
CCFAC	Codex Committee on Food Additives and Contaminants
CCGP	Codex Committee on General Principles
CCPR	Codex Committee on Pesticide Residues
CCRVDF	Codex Committee on Residues of Veterinary Drugs in Food
CE	Council of Europe
CIPAC	Collaborative International Pesticides Analytical Council Ltd
COREPER	Comite des Representants Permanents
EC	European Commission
ECB	European Chemical Bureau
ECCA	European Crop Care Association
ECDIN	Environmental Chemicals Data and Information of the European Communities
ECDIS	European Environmental Chemicals Data and Information System
ECE	Economic Commission for Europe

ECETOC	European Chemical Industry Ecology and Toxicology Centre
ECLO	Emergency Centre for Locust Operations
ECMWF	European Centre for Medium Range Weather Forecasting
ECPA	European Crop Protection Association
EDEXIM	European Database on Export an Import of Dangerous Chemicals
EHC (number)	Environment Health Criteria (number)
EHCD	Environmental Health Criteria Document
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINCS	European List of New Chemical Substances
EMIC	Environmental Mutagens Information Centre
EPA	Environmental Protection Agency
EPO	European Patent Office
EPPO	European and Mediterranean Plant Protection Organization
ESCORT	European Standard Characteristics of Beneficials Regulatory Testing
EU	European Union
EUPHIDS	European Pesticide Hazard Information and Decision Support System
EUROPOEM	European Predictive Operator Exposure Model
FAO	Food and Agriculture Organization of the UN
FOCUS	Forum for the Co-ordination of Pesticide Fate Models and their Use
FRAC	Fungicide Resistance Action Committee
GATT	General Agreement on Tariffs and Trade
GAW	Global Atmosphere Watch
GCOS	Global Climate Observing System
GCPF	Global Crop Protection Federation (formerly known as GIFAP)
GEDD	Global Environmental Data Directory
GEMS	Global Environmental Monotoring System
GIEWS	Global Information and Early Warning System for Food and Agriculture
GIFAP	Groupement International des Association Nationales de Fabricants de Produits Agrochimiques (now known as GCPF)
GRIN	Germplasm Resources Information Network
HRAC	Herbicide Resistance Action Committee
IARC	International Agency for Research on Cancer
IATS	International Academy of Toxicological Science
IBT	Industrial Bio-Test Laboratories
ICBB	International Commissin of Bee Botany
ICBP	International Council for Bird Preservation
ICES	International Council for the Exploration of the Seas
ICPBR	International Commissin for Plant-Bee Relationships
ILO	International Labour Organization
IMO	International Maritime Organisation
IOBC	International Organization for Biological Control of noxious Animals and Plants
IPCS	International Programme on Chemical Safety
IRAC	Insecticide Restistance Action Committee
IRC	International Rice Commission
ISCO	International Soil Conservation Orginazation
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry

JECFA	FAO/WHO Joint Expert Committee on Food Additives
JFCMP	Joint FAO/WHO Food and Animal Feed Contamination Monitoring Programme
JMP	Joint Meeting on Pesticdes (WHO/FAO)
JMPR	Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues (Joint Meeting on Pesticide Residues)
NATO	North Atlantic Treaty Organisation
NAFTA	North American Free Trade Agreement
NCI	National Cancer Institute (USA)
NCTR	National Center for Toxicological Research (USA)
NGO	non-governmental organization
NTP	National Toxicology Programme (USA)
OECD	Organization for Economic Cooperation and Development
OLIS	On-line Information Service of OECD
PAN	Pesticides Action Network
RNN	Re-registration Notification Network
RTECS	Registry of Toxic Effects of Chemical Substances (USA)
SCPH	Standing Committee on Plant Health
SETAC	Society of Environmental Toxicology and Chemistry
SI	Systeme International d`Unites
SITC	Standard International Trade Classification
TOXLINE	Toxicology Information On-line
UN	United Nations
UNEP	United Nations Environment Programme
WCDP	World Climate Data Programmme
WCP	Workd Climate Programme
WCRP	World Climate Research Programme
WFP	World Food Programme
WHO	World Health Organization
WTO	World Trade Organization
WWF	World Wide Fund for Nature

Appendix 2

Bacillus subtilis strain QST 713

Specific Terms and Abbreviations

B.10.2 Appendix II: Specific terms and abbreviations

Abb.	Definition
PAS	pure active substance
TAS	technical active substance