BASELINE DOSSIER

Bacillus subtilis QST 713

Microbial pest control agent against plant pathogenic fungi and bacteria

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

Summary documentation, Tier II

Annex IIM, Section 5

Point IIM 7: Fate and behaviour in the environment

Date: July 2015

Applicant

Bayer CropScience AG

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OWNERSHIP STATEMENT

Introduction

This document summarizes all data submitted for the initial evaluation of *Bacillus subtilis* QST 713 as an active substance under Directive 91/414. Data provided in the initial dossier and in subsequent additional submissions are listed chronologically under the respective data point according to the OECD dossier guidance (2006).

This document is further named as **"Baseline Dossier"** since it presents all data previously submitted.

IIM 7 Fate and behaviour studies on the Microbial Pest Control Agent in the environment

EU-Dossier: Doc M-IIB, Section 5

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 a viable organism being the active ingredient. 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.

IIM 7.1 Sufficient information on the origin, properties, survival and residual metabolites of the microorganism to assess its fate and behaviour in the environment. Viability/population dynamics, persistence, multiplication and mobility

Report: KIIM 7/01; [redacted]; [redacted]; [redacted]; [redacted]; 1999; M-528184-01-1
Title: Deliberate environmental release of bacteria
Report No.: M-528184-01-1
Document No.: M-528184-01-1
Guideline(s): --
Guideline deviation(s): --
GLP/GEP: **no**

EU-Dossier: Doc M-IIB, Point 7.1

With regard to environmental concern of the deliberate release of micro-organisms [redacted] et al. (1999b) state that basically the number of introduced organisms declines rapidly (sometimes after a brief period of proliferation) following application to both the soil and the field. The reviewed references mainly relate to survival in the *soil*: e.g. more cells survive in nutrient rich soils and in soils with increasing clay content. 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.

IIM 7.1.1 Persistence and mobility in soil

Report: KIIM 7.1.1/01; [redacted]; [redacted]; [redacted]; 1996; M-528072-01-1
Title: Persistence of Bacillus subtilis RB14 and its derivative strains in soil with respect to the LPA-14 gene
Report No.: M-528072-01-1
Document No.: M-528072-01-1
Guideline(s): --
Guideline deviation(s): --
GLP/GEP: **no**

Report: KIIM 7.1.1/02; [redacted]; [redacted]; [redacted]; [redacted]; 1986; M-153650-01-1
Title: Survival of Pseudomonas fluorescens and Bacillus subtilis introduced into two soils of different texture in field microplots
Report No.: M-153650-01-1
Document No.: M-153650-01-1
Guideline(s): --
Guideline deviation(s): --
GLP/GEP: **no**

Report:	KIIM 7.1.1/03; [redacted]; [redacted]; [redacted]; 1987; M-369240-01-1
Title:	Transfer of plasmid pFT30 between bacilli on soil as influenced by bacterial population dynamics and soil conditions
Report No.:	M-369240-01-1
Document No.:	M-369240-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

Report:	KIIM 7.1.1/04; [redacted]; [redacted]; 1973; M-497617-01-1
Title:	Growth of Bacillus subtilis and spore germination in soil observed by a fluorescent-antibody technique
Report No.:	M-497617-01-1
Document No.:	M-497617-01-1
Guideline(s):	not applicable
Guideline deviation(s):	not applicable
GLP/GEP:	**no**

Report:	KIIM 7.1.1/05; [redacted]; [redacted]; [redacted]; [redacted]; 1990; M-528278-01-1
Title:	Characteristics of Bacillus subtilis isolated from composts suppressing phytopathogenic microorganisms
Report No.:	M-528278-01-1
Document No.:	M-528278-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

Report:	KIIM 7.1.1/06; [redacted]; 1989; M-486913-01-1
Title:	Biological control of microbia plant pathogened - 1. introduction to plant pathology and microbial ecology
Report No.:	M-486913-01-1
Document No.:	M-486913-01-1
Guideline(s):	not applicable
Guideline deviation(s):	not applicable
GLP/GEP:	**no**

Report:	KIIM 7.1.1/07; [redacted]; 1997; M-528163-01-1
Title:	Final decision document: TSCA section 5 (H) (4) exemption for Bacillus subtilis
Report No.:	M-528163-01-1
Document No.:	M-528163-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

Report:	KIIM 7.1.1/08; [redacted]; [redacted]; [redacted]; [redacted]; [redacted]; 1994; M-001914-01-1
Title:	Guidance document on regulatory testing procedures for pesticides with non-target arthropods
Report No.:	Lit. 6841
Document No.:	M-001914-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

EU-Dossier: Doc M-IIB, Point 7.1.1 and Point 7.2

The phenomenon of fast decreasing vegetative cell numbers is reported for a *B. subtilis* strain introduced into soil ([redacted] et al., 1996), while in parallel sporulation increased. After a few days the cell population was shown to be stabilized as endospores. Van Elsas et al. (1986) 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 predominating sporulation at consistent 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 this difference to untreated soils was balanced. In studying the plasmid transfer between Bacilli in soil [redacted] et al. (1987) showed poor survival of vegetative *B. subtilis* cells in non-sterile soil compared to sterile soil or soil amended with bentonite clay, also survival in sterile soil was enhanced by nutrient addition.

[redacted] & [redacted] (1974) showed that *B. subtilis* cells added to acid forest soil did not grow unless *fungal* growth took place and concluded that the prime factor is nutrient supply; however an indirect effect of altered pH could not be excluded. [redacted] et al. (1990) 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.

Conclusively, application of organic matter, e.g. manure, will enhance growth of existing *B. subtilis* populations (and other bacteria) otherwise the cells will produce endospores.

Additionally, soil temperature was found to influence the survival of *B. subtilis*: [redacted] et al. (1996) ascribed temperature dependant survival of *B. subtilis* in soil to lower competition at higher temperatures (~ 30°C versus 15°C).

B. subtilis is an autochthonous soil micro-organism, the 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 ([redacted], 1989a, 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 ([redacted] et al., 1986).

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 falls, 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, as outlined below.

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

Serenade™ WP is applied to the foliage at a rate of 15 kg/ha in maximum and contains 5 x 10^9 cfu/g. An amount of 15 kg/ha will thus correspond to 7.5 x 10^{13} cfu/ha. Assuming the whole amount

would reach the soil surface uniformly, the resultant surface load would approximate 7.5 x 10^9 cfu/m^2 or ~ 7.5 x 10^5 cfu/cm^2. 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.

This still overestimated value can be regarded as low in view of the overall distribution of Bacilli in general, which occur at levels of 10^6 to 10^7 cfu/g (EPA, 1997) and considering the predominance of *B. subtilis* in all kinds of soils.

Employing a more realistic scenario under consideration of drift results in even lower levels of surface load:

According to Barret et al. (1994) a rate of 40% of the applied amount of product will reach the soil surface in three-dimensional crop, as orchards. Thus, one square cm of surface will receive a theoretical load of 3 x 10^5 cfu.

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.

Report:	KIIM 7.1.1/09; [redacted]; [redacted]; 1995; M-528856-01-1
Title:	Soil introductions of Bacillus subtilis as biocontrol agent and its population and acivity dynamic
Report No.:	M-528856-01-1
Document No.:	M-528856-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

Report:	KIIM 7.1.1/10; [redacted]; 1988; M-416655-01-1
Title:	Response in soil of cupriavidus necator and other copper-resistant - Bacterial predators of Bacteria to addition of water, soluble Nutrients, Various Bacterial Species, or Bacillus thuringiensis Spores and Crystals
Report No.:	M-416655-01-1
Document No.:	M-416655-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

Report:	KIIM 7.1.1/11; [redacted]; [redacted]; 1993; M-529054-01-1
Title:	Rhizosphere colonization of wheat by selected soil bacteria over diverse environments
Report No.:	M-529054-01-1
Document No.:	M-529054-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

Report:	KIIM 7.1.1/12; [redacted]; [redacted]; [redacted]; 2001; M-529208-01-1
Title:	Dominant fungi in the rhizosphere of established tea bushes and their interaction with the dominant bacteria under in situ conditions
Report No.:	M-529208-01-1
Document No.:	M-529208-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

Report: KIIM 7.1.1/13; [redacted]; [redacted]; [redacted]; 1992; M-368039-01-1
Title: Population dynamics of bacteriophage and bacillus subtilis in soil
Report No.: M-368039-01-1
Document No.: M-368039-01-1
Guideline(s): --
Guideline deviation(s): --
GLP/GEP: **no**

Report: KIIM 7.1.1/14; [redacted]; [redacted]; [redacted]; 1992; M-368050-01-1
Title: Survival of bacillus subtilis NB22, an antifungal-antibiotic iIturin producer, and its transformant in soil-systems
Report No.: M-368050-01-1
Document No.: M-368050-01-1
Guideline(s): --
Guideline deviation(s): --
GLP/GEP: **no**

Included under 3rd Additional Submission

Several studies ([redacted] & Gantchev, 1995; [redacted], 1988; [redacted] & [redacted], 1993; [redacted] et al. 2001; Pantastica-Caldas et al. 1992; [redacted] et al. 1993) were submitted in June 2002 and are cited in Addendum 1 to the Monograph (date of issue: 04.12.2002) They showed that populations of *B. subtilis* are influenced by biotic environmental factors. Introduced *B. subtilis* populations in the soil are subject to competition by the indigenous microflora (bacteria and fungi) and may also be affected by infectious agents like phages. As a result, high initial population numbers resulting from application of *Bacillus subtilis* will decline and reach a natural equilibrium.

As a conclusion of the ECCO Working Group Evaluation Meeting on 26.03.2003, it was stated that these data requirements are fulfilled.

IIM 7.1.2 Water

Report: KIIM 7.1.2/01; [redacted]; 1993; M-484952-01-1
Title: Systematics and ecology of Bacillus
Report No.: M-484952-01-1
Document No.: M-484952-01-1
Guideline(s): not specified
Guideline deviation(s): not specified
GLP/GEP: **no**

Report: KIIM 7.1.2/02; [redacted].; 1997; M-528163-01-1
Title: Final decision document: TSCA section 5 (H) (4) exemption for Bacillus subtilis
Report No.: M-528163-01-1
Document No.: M-528163-01-1
Guideline(s): --
Guideline deviation(s): --
GLP/GEP: **no**

EU-Dossier: Doc M-IIB, Point 7.1.2 and Point 7.2

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 ([redacted],

1993). However, *B. subtilis* is not regarded an autochthonous inhabitant of aquatic environments and does not find optimal conditions for growth, e.g. in waters 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.

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).

IIM 7.1.3 Air

Report:	KIIM 7.1.3/01; [redacted]; 1993; M-484952-01-1
Title:	Systematics and ecology of Bacillus
Report No.:	M-484952-01-1
Document No.:	M-484952-01-1
Guideline(s):	not specified
Guideline deviation(s):	not specified
GLP/GEP:	**no**

Report:	KIIM 7.1.3/02; [redacted]; 1997; M-528163-01-1
Title:	Final decision document: TSCA section 5 (H) (4) exemption for Bacillus subtilis
Report No.:	M-528163-01-1
Document No.:	M-528163-01-1
Guideline(s):	--
Guideline deviation(s):	---
GLP/GEP:	**no**

EU-Dossier: Doc M-IIB, Point 7.1.3 and Point 7.2

Endospores are suitable for aerial distribution as they are easily blown up with the wind ([redacted], 1993). Therefore, under conditions of use drift and 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 (dessication, UV-radiation, temperature), therefore survival of vegetative cells is limited (EPA, 1997).

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 (seizing with increasing distance), 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.

IIM 7.2 Other/special studies

No further studies have been performed.