Renewal
Assessment Report

Bacillus thuringiensis ssp. aizawai strain ABTS-1857

Volume 1

Rapporteur Member State: The Netherlands

Co-Rapporteur Member State: Germany

Version history

When	What
September 2018	Initial RAR

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Level 1

Active substance

1 Statement of subject matter and purpose for which this report has been prepared and background information on the application

1.1 Context in which the renewal assessment report was prepared

1.1.1 Purpose for which the renewal assessment report was prepared

The dossier for *Bacillus thuringiensis subsp. aizawai strain ABTS-1857* is submitted to support the renewal of approval of this micro-organism under Regulation 1107/2009/EC.

Also, this dossier contains data to support renewal of national authorizations of the formulations, i.e. plant protection products containing this active substance.

1.1.2 Arrangements between rapporteur Member State and co-rapporteur Member State

Not relevant.

1.1.3 EU Regulatory history for use in plant protection products

The following information about *Bacillus thuringiensis* ssp *aizawai* ABTS-1857 is available:
Review report: *Bacillus thuringiensis* ssp. *aizawai*, strain ABTS-1857, SANCO/1539/08 – rev. 4 d.d.13 December 2013.

It is mentioned on the commissions website the substance is fulfilling the criteria of Annex VI of Regulation (EC) 2229/2004: Criteria for clear indications of no harmful effects.

The substance has been included in Annex I of Directive 91/414/EC on 8 December 2008 (Directive 2008/113/EC).

Rapporteur member state of the first assessment for Annex I inclusion of Dir. 91/414/EC was Italy.

1.1.4 Evaluations carried out under other regulatory contexts

The active substance had been registered by US-EPA and by PMRA.

1.2 Applicant information

1.2.1 Name and address of applicant(s) for approval of the active substance

Applicant: Sumitomo Chemical Agro Europe S.A.S
Parc d'Affaires de Crécy
10A rue de la Voie Lactée
FR – 69370 Saint Didier au Mont d'Or

Contact Point: [redacted]

Phone: [redacted]

Fax: [redacted]

1.2.2 Producer or producers of the active substance

Confidential information: see Volume 4.

1.2.3 Information relating to the collective provision of dossiers

Sumitomo Chemical Agro Europe S.A.S was the sole applicant of the active substance, no task-force was formed.

1.3 Identity of the micro-organism

1.3.1 Name and species description, strain characterisation	BTa ABTS-1857
1.3.1.1 Composition of material used for manufacturing of the formulated product	
1.3.1.2 Accession number in culture collection	This culture has been placed in the Safe Deposit frozen storage facilities of the American Type Culture Collection (ATCC), Rockville, MD. Safe Deposit Number: SD-1372, 11 October 1990. This was converted to ATCC Patent Deposit 69074 on 7 July 1992.
1.3.1.3 Scientific name and taxonomic grouping, i.e. family, genus, species, strain, serotype, pathovar or any other denomination relevant to the micro-organism	
Taxonomy	Kingdom: bacteria Division: Firmicutes Class: Bacilli Family: Bacillaceae Genus: *Bacillus* Species: *Bacillus thuringiensis* Subspecies: *aizawai* Serotype: H7 Strain: ABTS-1857

	Bacillus thuringiensis was first described by Berliner in 1911 *Bacillus thuringiensis* subsp. *aizawai* was first described by Bonnefoi & de Barjac in 1963)
Indigenous or non-indigenous	Indigenous
Wild type	Yes
Spontaneous or induced mutant*	No
Genetically modified according to Directive 2001/18/EC*	No
* All known differences between the modified micro-organism and the parent wild strain must be provided	
1.3.1.4 Test procedures and criteria used for identification	
Monitoring growth characteristics on agar media, microscopical observations, biochemical analyses, plasmid profiles, Amplified fragment length polymorphism (AFLP), genomotyping and a rapid quantitative discriminatory PCR method	
1.3.1.5 Common name or alternative and superseded names and code names used during the development	American Type Culture Collection (ATCC) Number: SD-1372, 11 October 1990. This was converted to ATCC Patent Deposit 69074 on 7 July 1992.
1.3.1.6 Relationship to known pathogens	There are no records of the relationship to known plant or animal or human pathogens.
1.3.1.7 Method of manufacture (synthesis pathway) of the active substance	Confidential information, please refer to Volume 4.
1.3.2 Specification of the material used for manufacturing of formulated products	Confidential information, please refer to Volume 4.
1.3.3 Content of the micro-organism	In the formulated product XenTari® DF: Minimum 3 x 10^{10} CFU/g Nominal (Average) 5.5 x 10^{10} CFU/g Maximum 9 x 10^{10} CFU/g
1.3.4 Identity and content of impurities, additives, contaminating micro-organisms	
1.3.4.1 Significant impurities	None.
1.3.4.2 Relevant impurities	None.
1.3.4.3 Additives	None.
1.3.4.4 Contaminating micro-organisms	Complies with SANCO/12116/2012 rev. 0
1.3.5 Analytical profile of batches	Please refer to Volume 4

1.4 Information on the plant protection product

1.4.1 Applicant	Sumitomo Chemical Agro Europe S.A.S Parc d'Affaires de Crécy 10A rue de la Voie Lactée FR – 69370 Saint Didier au Mont d'Or
1.4.2 Producer of the plant protection product	Confidential information. Please refer to volume 4.
1.4.3 Current, former and proposed trade names and development code numbers	
Trade Name	XenTari® WG Other trade names: Florbac®, XenTari®, and Xentari® GD
Code Number	BTa ABTS-1857 The product formulation with code reference ABG-6314 is identical to the plant protection product formulation XenTari® WG.
1.4.4 Detailed quantitative and qualitative information on the composition of the plant protection product	
1.4.4.1 Composition of the plant protection product	XenTari® WG contains 54% w/w active substance (15,000 IU/mg or 35,000 DBM Units/mg). The identity and content of the composition, co-formulants, impurities and additives is confidential to Valent Biosciences Corp. and is presented in volume 4.
1.4.4.2 Information on the active substances	The active substance is Bacillus thuringiensis ssp. aizawai (strain ABTS-1857) (fermentation solids, spores and insecticidal toxins).
1.4.4.3 Information on safeners, synergists and co-formulants	The identity and content of the composition, co-formulants, impurities and additives is confidential to Valent Biosciences Corp. and is presented in volume 4.
1.4.5 Type and code of the plant protection product	Formulation type: Water Dispersible Granule (WG).
1.4.6 Function	insecticide
1.4.7 Field of use envisaged	For the use as a insecticide by spraying in outdoor fruit vegetables (pepper) and indoor fruit vegetables (pepper)
1.4.8 Effects on harmful organisms	Non-systemic, biological insecticide, curative, triggered by ingestion Target organisms are leaf eating caterpillars belonging to the order of Lepidoptera

1.5 Detailed uses of the plant protection product

1.5.1 Details of representative uses

Crop and/or situation (a)	Member State or EU region	Product code	F, G or I (b)	Pests or Group of pests controlled (c)	Formulation		Application				Application rate per treatment			PHI (days) (l)	Remarks (m)
					Type (d-f)	Conc. of a.s.	Method (f-h)	Growth stage BBCH (j)	Number of applications (k)	Min. interval between applications (Days)	Kg a.s. /hL	Water (L/ha)	Use rate Kg a.s. /ha		
											min – max	min – max	min-max		
Outdoor fruit vegetables (pepper)	CEU / SEU	XenTari® WG (ABG-6314)	F	Lepidoptera larvae including but not limited to: HELIAR, HELISP, LAPHEG, PYRUNU, SPODLI, GNORAB, SPODSP.	WG (Water Dispersible Granules)	54% w/w Approx. $5x10^{13}$ cfu/kg	Spray	BBCH 09-BBCH 92 (May-Oct) Start when larvae hatch (L1)	1 – 8, (1 - 3 per generation o pest)	6 - 10 Typically 7 days	0.027 – 0.054 kg a.s. /hL (0.050 – 0.100 kg f.p./hL)	Typically 500-1000 L/ha	0.270– 0.540 kg a.s./ha. (0.500-1.0 kg f.p./ha) Approx. $5x10^{13}$ cfu/ha	-	Increase dose rates at high pest pressure, and mixed populations with older larvae. Regarding water volume, typically 500-1,000 L/ha to ensure full coverage but not to the point of run off.
Indoor fruit vegetables (pepper)	EU	XenTari® WG (ABG-6314)	G	Lepidoptera larvae including but not limited to: 1NOCTF GNORAB	WG (Water Dispersible Granules)	54% w/w Approx. $5x10^{13}$	Spray	BBCH 09-BBCH 92 (Jan-Dec)	1 – 7, Typically (1 - 3 per generation o pest)	Typically 7 days	0.027 – 0.054 kg a.s./hL	400– 1000 Typically 1000 L/ha	0.270 – 0.540 kg a.s./ha.	-	Increase dose rates at high pest pressure, and mixed populations with older lar-

Crop and/or situation (a)	Member State or EU region	Product code	F, G or I (b)	Pests or Group of pests controlled (c)	Formulation		Application				Application rate per treatment			PHI (days) (l)	Remarks (m)
					Type (d-f)	Conc. of a.s.	Method (f-h)	Growth stage BBCH (j)	Number of applications (k)	Min. interval between applications (Days)	Kg a.s. /hL	Water (L/ha)	Use rate Kg a.s. /ha		
											min – max	min – max	min-max		
				HELIAR LAPHEG PLUSCH SPODLI SPODSP HELISP		cfu/kg		Start when larvae hatch (L1)			(0.050-0.100 kg f.p./hL)		(0.500–1.0 kg f.p./ha) Approx. $5x10^{13}$ cfu/ha		vae. Regarding water volume typically 1,000 L/ha to ensure full coverage but not to the point of run off.

1.5.2 Further information on representative uses

The applicant claims a variable dose rate (0.027-0.054 kg/hL) the higher rates are intended for high pest pressure, and mixed populations with older larvae.
Growth stage BBCH 09-92 (May-October) and starts when the larvae hatch (L1)
1-8 applications per crop (1-3 per generation of pest)
Minimum interval is 6 days
Water volume is 400-1000 L/ha to ensure full coverage but not to the point of run off.

1.5.3 Details of other uses applied for to support the setting of MRLs for uses beyond the representative uses

No other uses were submitted.

1.5.4 Overview on authorisations in EU Member States

Country	Status	Trade name	A.S. Content	Reg. Number	Registered Uses
Austria	**Registered**	XenTari	540 g/kg	3431	Vegetables; cabbages; grapes; ornamentals; stine fruits; pome fruits; berries
Austria	**Registered**	Florbac	540 g/kg	3431-901	Vegetables; cabbages; grapes; ornamentals; stine fruits; pome fruits; berries
Belgium	**Under evaluation**	Xentari WG	540 g/kg	9067P/B	Indoor and outdoor vegetables; Outdoor Brassica; pome fruits; grapes; indoor /outdoor ornamentals; trees; sweet corn;
France	**Registered**	Xentari WG	540 g/kg	2020241	Indoor and outdoor vegetables; Outdoor Brassica; pome fruits; grapes; indoor /outdoor ornamentals; aromatic plants; grapes; general treatment; olives; rice
Germany	**Registered**	XenTari	540 g/kg	024426-00	Vegetables; cabbages; grapes; ornamentals; stone fruits; pome fruits; berries
Greece	**Under evaluation**	Xentari WG	540 g/kg	1798	Indoor and outdoor vegetables; Outdoor Brassica; pome fruits; stone fruits;olives; grapes; indoor /outdoor ornamentals; rice

Italy	**Under evaluation**	Xentari WG	540 g/kg	011793	Indoor and outdoor vegetables; Outdoor Brassica; pome fruits; stone fruits; grapes; indoor /outdoor ornamentals; olives; rice
Netherlands	**Registered**	Xentari WG	540 g/kg	12437N	Indoor and outdoor vegetables; Outdoor Brassica; pome fruits; grapes; indoor /outdoor ornamentals; strawberry; berries; tree nursery
Netherlands	**Registered**	Florbac	540 g/kg	15033N	Indoor and outdoor vegetables; Outdoor Brassica; pome fruits; grapes; indoor /outdoor ornamentals; strawberry; berries; tree nursery
Spain	**Under evaluation**	Xentari GD	540 g/kg	19692	Indoor and outdoor vegetables; Outdoor Brassica; pome fruits; grapes; indoor /outdoor ornamentals; grapes; pome fruits; stone fruits; olives; rice

Level 2

Bacillus thuringiensis ssp. aizawai strain ABTS-1857

2 Summary of active substance hazard and of product risk assessment

2.1 Identity

This document is an assessment of the active substance *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS-1857) and the plant protection product XenTari® WG. *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS-1857) is deposited in the Safe Deposit storage facilities of the American Type Culture Collection, Rockville, MD under the identification number ATCC-SD-1372. A new genomotyping study is available to unequivocally identify the organism down to strain level.

2.2 Biological properties

2.2.1 Summary of biological properties of the active substance

XenTari® WG is a water dispersible granule (WG) formulation containing 54% w/w MPCA equivalent to 15,000 IU/mg *Bacillus thuringiensis* subsp. *aizawai*, strain ABTS-1857. The formulation is a fine, light brown granular solid with a bulk density of 0.383 g/mL. It is not flammable and does not have any explosive or oxidizing properties. The pH of 1% dilution is 4.7. The product is characteristic of a free flowing solid and has been shown to be stable for at least 24 months at 25ºC when stored in HDPE or composite cans packaging. Storage under frozen conditions (-18ºC) did not affect potency. The product is not known to have any physical-chemical properties of technical or hazardous concern and its technical properties are acceptable for an WG-formulation.

Bacillus thuringiensis ssp. *aizawai* strain ABTS-1857 has an insect-specific mode of action, via the production of crystalline proteinaceous inclusions containing the δ-endotoxins Cry1Aa, Cry1Ab, Cry1C and Cry1D. These endotoxins are broken down in the environment of the mammalian gastrointestinal tract and, consequently, are without effect in mammalian species. The endotoxins are demonstrated to be broken down by protease enzymes in the environment of the mammalian gastrointestinal tract and, consequently, are without effect in mammalian species. A study *in vitro* in bovine and porcine gastrointestinal cells and a human cell line with the model δ-endotoxin Cry1Ab indicate a low level of non-specific binding but no effects on cell function or viability, even following prolonged incubation. Findings in mammalian cells are in contrast to the marked effects on the function and viability of insect gastrointestinal cells. The specificity of Bt endotoxins is also demonstrated in a sub-chronic dietary study with genetically modified maize encoding Cry1AB (at a dietary concentration of 33%). This study did not show any effects of treatment. A further sub-chronic toxicity study also shows an absence of adverse effects of Cry1Ab when administered to rats with experimentally-induced gastrointestinal impairment.

Bacillus thuringiensis is closely related to *Bacillus cereus*, a species associated with food poisoning outbreaks. *B. cereus* food poisoning is associated with symptoms of diarrhoea and vomiting; and is due to the production (by some *B. cereus* strains) of the diarrhoeal enterotoxins haemolysin BL, non-haemolytic enterotoxin and cytotoxin K; and the emetic toxin cereulide.

Some strains of *B. thuringiensis* have also been shown to produce diarrhoeal enterotoxins; however none produce the emetic toxin cereulide. *Bacillus thuringiensis* ssp. *aizawai* strain ABTS-1857 does not produce enterotoxins under the conditions of manufacture. This strain has been shown to produce low levels of enterotoxin under very specific culture conditions not representative of those used in manufacture and extremely unlikely to be encountered following product application. The risk of food poisoning due to this strain is

therefore considered to be negligible. Due to the very close similarity of *B. cereus* and *B. thuringiensis*, routine analyses in food poisoning cases cannot distinguish the two species. The Bacillus cereus group contains vertebrate pathogens such as B. anthracis and B. cereus and the invertebrate pathogen B.thuringiensis (Bt). Microbial biopesticides based on Bt are widely recognised as being among the safest and least environmentally damaging insecticidal products available. Nevertheless, a recent food-poisoning incident prompted a European Food Safety Authority review which argued that Bt poses a health risk equivalent to B. cereus, a causative agent of diarrhoea. However, a critical examination of available data, and this latest incident, provides no solid evidence that Bt causes diarrhoea. Although relatively high levels of B. cereus-like spores can occur in foods, genotyping demonstrates that these are predominantly naturally occurring strains rather than biopesticides. More-over, MLST genotyping of >2000 isolates show that biopesticide genotypes have never been isolated from any clinical infection. MLST data demonstrate that B. cereus group is heterogeneous and formed of distinct clades with substantial differences in biology, ecology and host association. The group posing the greatest risk (the anthracis clade) is distantly related to the clade containing all biopesticides. These recent data support the long-held view that Bt and especially the strains used in Bt biopesticides are very safe for humans.

Strain ABTS-1857 was sensitive to gentamicin, kanamycin, erthyromycin, clindamycin, vancomycin, chloramphenicol and trimethoprim/sulfamethoxazole but not sensitive to penicillin, ampicillin or cephalothin. The susceptibility was determined using standard methodology (SOP 047T-12-043A; National Committee for Clinical Laboratory Standard, 1984). *Staphylococcus aureus* was also included in the test to verify the test procedure (Smith, 1990). Although for the assessment of antibiotic resistance in the EU it would be preferable to use broth dilution minimum inhibitory concentration (MIC) methodology against relevant antibiotics since acceptable MICs specifically for Bacillus ssp. have been established for regulatory use in the EU whereas no acceptance criteria exist for the NCCLS method (no definition of microbiological breakpoints (criteria for sensitivity) exist in the EU for regulatory purposes) the conclusion that the strain possesses 3 resistances has been supported by the RMS at the moment. The guidance to address this specific data requirement is in development in the EU.

2.2.2 Summary of physical, chemical and technical properties of the plant protection product

XenTari® WG is a water dispersible granule (WG) formulation containing 54% w/w MPCA equivalent to 15,000 IU/mg Bacillus thuringiensis subsp. aizawai, strain ABTS-1857. The formulation is a fine, light brown granular solid with a bulk density of 0.383 g/mL. It is not flammable and does not have any explosive or oxidizing properties. The pH of 1% dilution is 4.7. The product is characteristic of a free flowing solid and has been shown to be stable for at least 24 months at 25ºC when stored in HDPE or composite cans packaging. Storage under frozen conditions (-18ºC) did not affect potency. The product is not known to have any physical-chemical properties of technical or hazardous concern and its technical properties are acceptable for an WG-formulation.

2.3 Data on application and efficacy

2.3.1 Summary of effectiveness

According to the latest guidance on the preparation of dossiers for the renewal of active substances, information on efficacy is not required (SANCO/10181/2013 – rev. 2.1, 13 May 2013). The representative products have all been authorised at Member State level for > 10 years and have therefore been assessed in line with Uniform Principles. It can therefore be concluded that the dose rates for the representative uses are realistic.

2.3.2 Summary of information on the development of resistance

Bacillus thuringiensis subsp. *aizawai* Strain ABTS-1857 is a microbial disruptor of insect midgut membranes. As with any insecticide, too frequent use of one type of *Bt* strain or one type of *Bt* delta-endotoxin can result in resistance of the insect to the active ingredient. *Bacillus thuringiensis* is a biological non-persistent insecticide thus reducing the chances of resistance build up. No cross-resistance has been reported between chemical insecticides and *Bt* products (Sarnthoy *et al.*, 1997: Smirle *et al.*, 2003). Certain insect species have developed a resistance to particular *Bt* products caused by prolonged use resulting in a reduction in binding of specific Cry toxins to the gut membrane binding site. However, indications are that certain pest species are susceptible to more than one Cry toxin produced by different *Bt* subspecies. Therefore, resistance management strategy of altering applications of *Bt* products can prove effective.

In conclusion, *Bt* products like any other insecticide should be used in IRM (Insecticide Resistance Management) or IPM (Integrated Pest Management) programs and not used over and over as the only insecticide of choice. IRM and IPM cultural practices are commonly in place already.

While resistance to *Bacillus thuringiensis* does occur, it can be concluded that the proposed GAP for the representative uses is still realistic. Resistance management will have to be evaluated by member-states during product renewal or authorisation, as it can depend on local resistant populations, agricultural practices and other variables.

2.3.3 Summary of adverse effects on treated crops

The representative products have all been authorised at Member State level for > 10 years and have therefore been assessed in line with Uniform Principles. For the purpose of renewal of the active substance no new information is required.

2.3.4 Summary of observations on other undesirable or unintended side-effects

The representative products have all been authorised at Member State level for > 10 years and have therefore been assessed in line with Uniform Principles. For the purpose of renewal of the active substance no new information is required.

2.4 Further information

2.4.1 Summary of methods and precautions concerning handling, storage, transport or fire

Hazard symbols: None

Indications of danger: None

Risk phrases: None

Safe handling: Store in a dry and cool place. Keep container in a well-ventilated place. Keep away from food, drink and animal feeding stuffs. Do not drink, eat and smoke in work areas. Other information. Do not mix with water (except for the normal preparation).

2.4.2 Summary of procedures for destruction or decontamination

Dispose of contents/container in accordance with local regulation.

2.4.3 Summary of emergency measures in case of an accident

Containment of a spill; Do not allow to escape into sewage system or water courses.
Clean-up procedures; Clean up spills immediately. Sweep up and place into sealable containers. Dig up heavily contaminated soil and place into drums. Use a damp cloth to clean floors and other objects, and also place in sealable container. Dispose of all waste and contaminated clothing in the same manner as waste chemicals (i.e. via an authorized disposal facility). Do not wash residues into drains or other waterways.

2.5 Analytical methods

Genomotyping which makes analysis of bacteria by comparison of their genomes using microarrays and a rapid quantitative discriminatory PCR method allow unequivocal identification.

The activity of Btk essentially depends on the content of the crystal proteins, in particular the Cry 1Ab protein. Several methods have been used to quantify Cry1Ab including enzyme linked immunosorbent assay (ELISA), sodium dodecyl sulfate polyacrylamide gel electrophoresis densiometry (SDS-PAGE) and total protein assays such as the Bradford assay. The validation presented shows that quantification of Cry1Ab by SDS-PAGE is the preferred method and can be used as a monitoring method to identify Cry1Ab.

The strains may produce enterotoxins, β-exotoxin and cytolytic proteins and these may be present in the technical material. Methods exist for the determination of contaminants during the production process such as an HPLC-UV and a House Fly Bioassay for the determination of β-exotoxin and a TECRA Bacillus Diarrhoeal Enterotoxin Visual Immunoassay for the detection of soluble enterotoxin protein in production beers and finished products of Bacillus thuringiensis.

Microbial contaminant screenings were carried out following standard microbiological methods which are considered validated as such.

A bioassay based on the quantal dose response of four-day post-hatch *Trichoplusia ni* larvae to the test substance incorporated into an agar-based diet has been validated as a method to determine the biopotency of the preparation. The study meets the requirements of Part B of Regulation (EU) No 284/2013. The method was used to determine the storage stability and shelf life of the preparation.

Post-registration monitoring methods are different only in the isolation method that depends on the medium that is being analysed. The methods used for subsequent determination and quantification of residues are independent from the source of the isolates. A combination of three techniques; plate counting on agar plates, SDS-PAGE and PCR amplification have been validated and can be used for post-monitoring purposes. Bta ABTS-1857, as all other Bt strains currently registered at EU level, was proposed for inclusion into Annex IV of Regulation (EC) No 396/2005. This means that no residue definition applies to the microorganism and no MRL is set for any of the existing or intended uses. This issue, however, is still under discussion (see B.7). No specific MRL was fixed for the active substance under Reg. (EC) No 396/2005, according to Art. 18(1)(b) of that Regulation. Up till now Bacillus thuringiensis subsp. aizawai strain ABTS-1857 is not included in Annex IV due to delay at EFSA. Moreover, the default MRL of 0.01 mg/kg is not

applicable because agencies are not used to follow enforcement or maintenance procedures for micro-organisms. Furthermore, the evaluation of the renewal to still going on to disprove the EFSA opinion that *Bacillus thuringiensis* subsp. *aizawai* strain ABTS-1857 and pathogenic B. cereus strain are comparable. If commercial *B. thuringiensis* strains such as *B. thuringiensis* ABTS-1857 are assessed in a different way as other Bacillus spp. in foods, the validation of strain specific analytical methods for the determination of residues in plants is required according to the data requirements in SANCO/825/00 rev. 8.1.

Therefore, methods for the determination and quantification of residues are currently not required. However, strain specific markers are available which can be used for monitoring of the strain upon field application.

2.6 Impact on human and animal health

2.6.1 Effects having relevance to human and animal health arising from exposure to the micro-organism or to impurities, additives, contaminating micro-organisms contained in the material used for manufacturing of formulated products

Bacillus thuringiensis is closely related to *Bacillus cereus*, a species associated with food poisoning outbreaks. *B. cereus* food poisoning is associated with symptoms of diarrhoea and vomiting; and is due to the production (by some *B. cereus* strains) of the diarrhoeal enterotoxins haemolysin BL, non-haemolytic enterotoxin, PlcR and cytotoxin K; and the emetic toxin cereulide.

Some strains of *B. thuringiensis* have also been shown to produce diarrhoeal enterotoxins; however none produce the emetic toxin cereulide. *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857) does not produce enterotoxins under the conditions of manufacture. This strain has been shown to produce low levels of enterotoxin under very specific culture conditions not representative of those used in manufacture and extremely unlikely to be encountered following product application. The risk of food poisoning due to this strain is therefore considered to be negligible. Due to the very close similarity of *B. cereus* and *B. thuringiensis*, routine analyses in food poisoning cases cannot distinguish the two species. It is noteworthy that in the EFSA Scientific Opinion it is noted that no definitive demonstration has been provided for the actual role of the enterotoxins (alone or in combination) in the diarrheal syndrome. The evidence does not therefore implicate the use of biopesticides strains such as *Bacillus thuringiensis* ssp. *aizawai (*strain ABTS 1857) as a cause of food poisoning.

Occupational exposure

Routine medical surveillance of manufacturing and formulation plant workers over a number of years shows no evidence of adverse health effects attributable to *Bacillus thuringiensis* ssp. *aizawai (*strain ABTS 1857). There is no evidence for health effects resulting from the widespread application of *B. thuringiensis* biopesticides (*Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857) in forestry, agricultural or urban areas.

Sensitisation

Bacillus thuringiensis ssp. *aizawai* (strain ABTS 1857) showed no evidence of skin sensitisation in a guinea pig study. However as a general policy within the EU it is considered that, regardless of the supporting data, all microbial active substances are potential skin and respiratory sensitisers. A study of greenhouse workers exposed to biopesticides showed the presence of specific IgE in those exposed to *B. thuringiensis* based

products; however there was no effect on the incidence or prevalence of respiratory symptoms, lung function or bronchial responsiveness. The potential for sensitisation is therefore concluded to be minimal.

Acute toxicity, pathogenicity and infectivity

Bacillus thuringiensis ssp. *aizawai* (strain ABTS 1857) is of low toxicity following acute oral, inhalation, intratracheal, intraperitoneal, subcutaneous and intravenous exposure. Studies using acute oral, intratracheal, intraperitoneal, subcutaneous and intravenous administration show a lack of pathogenicity and infectivity.

Genotoxicity

Studies of genotoxicity *in vitro* are not available for *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857) in the absence of any validated methods for the assessment of the genotoxicity of microorganisms. Studies of repeated exposure inhalation toxicity are not available and are not considered to be required for *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857) based on the lack of evidence for toxicity, pathogenicity or infectivity in acute studies. Published studies report no toxicologically significant findings following the sub-chronic oral administration of genetically modified maize encoding Cry1AB protein or following the sub-chronic oral administration of purified Cry1AB protein.

A number of published studies are available which address the *in vivo* genotoxicity of *B. thuringiensis* spore proteins. A bone marrow micronucleus study in the mouse using oral administration of spore-crystal endotoxins Cry1IA, Cry10Aa and Cry1Ba6 reports an absence of genotoxicity. Administration of *B. thuringiensis* ssp. *kurstaki* spore crystals Cry1Aa, Cry1Ab, Cry1Ac or Cry2Aa to the mouse is reported by the authors to have caused haematological effects but not to have induced micronucleus formation in the bone marrow. This study is not considered to be reliable due to deficiencies in methodology, reporting and data interpretation. The same authors report increased incidences of micronuclei in groups of mice administered the same *B. thuringiensis* ssp. *kurstaki* spore crystals by intraperitoneal injection; findings are associated with effects on haematological parameters and evidence of direct bone marrow toxicity. The results of this study should be considered in light of other (unreliable) data reported by the same group but, in any case, are not considered to raise concerns of genotoxicity following exposure by a physiological route.

Repeated inhalation exposure toxicity

No data are available; however there is no evidence of toxicity, pathogenicity or infectiveness from acute inhalation studies. Repeated dose oral toxicity studies with Cry1Ab and with other *B. thuringiensis* strains do not show any effects at very high dose levels. Based on the absence of effects in animal studies and the absence of effects in exposed workers, there is therefore no concern relating to repeated inhalation exposure to *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857).

Summary and conclusions of Tier I studies

Study	Vehicle	Dose levels	Results
Acute oral toxicity/pathogenicity study of *Bacillus thuringiensis* ABG-6305 in rats	*Bacillus thuringiensis* ABG-6305	gavage: 10 ml of 7.9 x 10^7 CFU/ml	No signs of infectivity, toxicity or pathogenicity
XenTari Technical Powder (ABG-6305): Acute oral toxicity study in rats	ABG-6305 Technical powder	gavage: 5050 mg/kg (approximately 6 x 10^9 CFU), dose volume of 16.8 ml/kg	LD_{50} >5050 mg/kg bw

XenTari Technical Powder (ABG-6305): Acute inhalation toxicity study in rats	XenTari Technical Powder	aerosol at a concentration of 5.33 mg/L (3.9 x 108 CFU/L) for 4 hours	LC50 >5.33 mg/L
Acute pulmonary toxicity and infectivity/pathogenicity to rats of Bacillus thuringiensis ABG-6305	*Bacillus thuringiensis* ABG-6305	Intratracheal: 5.33 x 108 CFU/ml (108 CFU/animal	mild toxicity and no pathogenicity or infectivity Complete clearance was seen at Day 35
Intraperitoneal and subcutaneous injection tests with ABG-6305 Technical Powder	ABG-6305 Technical Powder	Subcutaneous / intraperitoneal: 0.005, 0.05 and 0.5 mg/animal equivalent to 1 x 10^6, 1 x 10^7 and 1 x 10^8 CFU/animal	no signs of toxicity, pathogenicity or infectivity
Acute intravenous toxicity and infectivity/pathogenicity to rats of ABG-6305	*Bacillus thuringiensis* ABG-6305	2.94 x 10^7 cfu/ml at a dose rate of 3.0 ml/kg (1 x 10^7 CFU/mL)	mild clinical signs of toxicity (piloerection) but no signs of pathogenicity or infectivity were observed

Other studies

A published study of the effects of Xentari® WG (containing *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857) reports a reduction in the numbers of implantation numbers and marked histopathological changes in the uterus, following repeated gavage administration on Gestation Days 0-7. The dose level used in this study (3500 mg/kg bw/d) exceeds the regulatory limit dose by a large margin; the authors also report very similar effects for a deltamethrin product, which is not characteristic of the (well characterised) toxicity of this substance. The results of this study are considered to be unreliable and not of relevance to the human risk assessment.

The same group also report marked effects of Xentari® WG on litter size in pregnant rats and histopathology of the kidneys, liver and lungs following repeated gavage administration on Gestation Days 0-7. The dose levels used in this study (up to 3700 mg/kg bw/d) exceed the regulatory limit dose by a large margin; the authors also report very similar effects for a deltamethrin product, which is not characteristic of the (well characterised) toxicity of this substance. The findings of this study are inconsistent with the toxicological dataset and indicate severe lung effects secondary to dosing accidents at the highest dose level. The results of this study are considered to be unreliable and not of relevance to the human risk assessment.

2.6.2 Impact on human health arising from exposure to the micro-organisms or to impurities, additives, contaminating micro-organisms contained in the material used for manufacturing of formulated products

The product XenTari® WG (development code ABG-6314) is of low acute oral, dermal and inhalation toxicity. An acute oral LD_{50} of >5000 mg/kg bw is reported in a study in the rat; an acute dermal LD_{50} of >2000 mg/kg bw is reported in a study in the rabbit. No mortality was reported at the maximum attainable concentration of 3.05 mg/L in an acute inhalation toxicity study with XenTari WG. An acute inhalation toxicity study performed with the related product XenTari DF reports an LC_{50} value of 5.10 mg/L. XenTari WG does not therefore require classification for acute toxicity according to the CLP Regulation. XenTari WG is a slight skin irritant, but does not require classification for skin irritation according to the CLP Regulation. Findings in an eye irritation study trigger classification of XenTari WG as an eye irritant according to the CLP Regulation. No evidence of skin sensitisation was seen in a Maximisation study. Classification of XenTari WG as a skin sensitiser is not required

according to the CLP Regulation; however given the microbial nature of the active substance the product is considered to be a potential sensitiser. The product label must include a standard warning phrase and dermal and inhalation exposure should be minimised through the use of appropriate PPE. The standard warning phrase may need to be reviewed as weight of evidence of years of use of XenTari WG has not presented any sensitization incidents.

Bacillus thuringiensis ssp. *aizawai* (ABTS 1857) is not toxic, infective or pathogenic. The product XenTari WG is of low toxicity but is considered to be a potential skin and respiratory sensitiser. Commercial products manufactured from different strains of *Bacillus thuringiensis* have been applied in agriculture and forestry for crop and tree protection and in waterways for public health over many decades and very few health concerns relating to its use have ever been reported. Despite the widespread use of *Bacillus thuringiensis*-based products, the instances of allergic reaction following exposure are also considered to be very rare.

2.6.3 Summary of product exposure and risk assessment

Derivation of toxicological reference values (including an AOEL) is not applicable for *Bacillus thuringiensis* ssp. *aizawai* (ABTS 1857) based on the lack of toxicity, infectivity or pathogenicity. Quantitative assessment of operator, worker, bystander and resident exposure is therefore not required for the product XenTari WG. It is therefore concluded that the product can be used without potential health risks to operators, workers or bystanders, subject to the use of protective equipment specified on the product label

2.7 Residues in or on treated products, food and feed

2.7.1 Persistence and likelihood of multiplication in or on crops, feedstuffs or foodstuffs

B. thuringiensis is naturally occurring in the environment and has been isolated from a range of habitats. BTa ABTS-1857 shows a low acute toxicity via the oral and dermal routes. *B. thuringiensis* has been found to have short residues persistence on foliage, with insecticidal activity declining rapidly within one day.

The occurrence of *Bacillus* species in food is generally below 10^5 cells/g or ml. Although there is some limited evidence in literature that use of bio-insecticide products may lead to "residues" of *B. thuringiensis* on treated crops, there is no evidence that *B. thuringiensis* can grow and multiply on food and the levels found are no higher than those of naturally occurring ubiquitous *B. cereus*. There is strong evidence to demonstrate that *B. thuringiensis* does not grow and multiply in or on food either when treated with commercial pesticide products or during subsequent processing.

Residue studies on lettuce**,** tomatoes and peppers conducted at rates equivalent to or greater than the maximum individual dose rate per treatment have been conducted. Residues of spores were shown to decline between applications, meaning that the final application has the most significant impact on the final residue level. Treated were not found to contain spore concentrations greater than 1×10^5 CFU/g fresh weight, with the exception of one pepper sample where levels were found very close to 1×10^5 CFU/g fresh weight. It can be concluded therefore that concentrations of spores will be significantly below 1×10^5 CFU/g fresh weight following treatment of peppers with BTa ABTS-1857 according to the proposed GAP.

Sufficient evidence is available to demonstrate that BTA ABTS-1857 is of low risk to humans. BTa

ABTS-1857 is not a human pathogen but is related to the human pathogen *Bacillus cereus*. There is no evidence for enterotoxin production under normal growth conditions for BTa ABTS-1857. Even if enterotoxin production were to occur, which is unlikely as the optimum conditions for spore growth an enterotoxin production are not available, any enterotoxin produced will be at significantly lower levels than those produced by known pathogenic *B. cereus* strains. Furthermore, studies showed no germination or growth of vegetative cells through a simulated gut therefore there is limited possibility for cells to survive when exposed to gastric acid and bile during in-vitro gastrointestinal transit.
The occurrence of *Bacillus* species in food is generally below 10^5 cells/g or ml. Residues trials data demonstrate spore counts of BTa ABTS-1857 do not exceed this level when crops are treated according to the proposed GAP.
These data combined with the ubiquitous nature of *B. thuringiensis* provides a weight of evidence supporting the non-pathogenic nature of *B. thuringiensis* to humans. The presence of BTa ABTS-1857 in foodstuffs following its use as a plant protection product is of no risk and a consumer risk assessment is not required. Neither a residue definition nor MRLs are considered necessary.

2.7.2 Further information required

2.7.2.1 Non-viable residues

Non-viable residues do not pose a risk to humans or the environment. Crystal proteins, the other major component in commercial Bt preparations apart from spores, are not toxic to mammals as indicated in different publications. In addition, crystal proteins are very unstable when exposed to light.

2.7.2.2 Viable residues

The occurrence of *Bacillus* species in food is generally below 10^5 cells/g or ml. Although there is some limited evidence in literature that use of bio-insecticide products may lead to "residues" of *B. thuringiensis* on treated crops, there is no evidence that *B. thuringiensis* can grow and multiply on food and the levels found are no higher than those of naturally occurring ubiquitous *B. cereus*. There is strong evidence to demonstrate that *B. thuringiensis* does not grow and multiply in or on food either when treated with commercial products or during subsequent processing.

Residue studies on lettuce**,** tomatoes and peppers conducted at rates equivalent to or greater than the maximum individual dose rate per treatment have been conducted. Residues of spores were shown to decline between applications, meaning that the final application has the most significant impact on the final residue level. Treated commodities were not found to contain spore concentrations greater than 1 x 10^5 CFU/g fresh weight, with the exception of one pepper sample where levels were found very close to 1 x 10^5 CFU/g fresh weight. It can be concluded therefore that concentrations of spores will be significantly below 1 x 10^5 CFU/g fresh weight following treatment of peppers with BTa ABTS 1857 according to the proposed GAP. Therefore, RMS concluded that the occurrence of Bacillus species in food is generally below 10^5 cells/g or ml based on the residues trials data demonstrating that spore counts of BTa ABTS-1857 do not exceed this level when crops are treated according to the proposed GAP.

Sufficient evidence is available to demonstrate that BTA ABTS-1857 is of low risk to humans. BTa ABTS-1857 is not a human pathogen but is related to the human pathogen *Bacillus cereus*. There is no evidence for enterotoxin production under normal growth conditions for BTa ABTS-1857. Even if

enterotoxin production were to occur, which is unlikely as the optimum conditions for spore growth an enterotoxin production are not available, any enterotoxin produced will be at significantly lower levels than those produced by known pathogenic *B. cereus* strains. Furthermore, studies showed no germination or growth of vegetative cells through a simulated gut therefore there is limited possibility for cells to survive when exposed to gastric acid and bile during in-vitro gastrointestinal transit.

These data combined with the ubiquitous nature of *B. thuringiensis* provides a weight of evidence supporting the non-pathogenic nature of B. thuringiensis to humans.

No specific MRL was fixed for the active substance under Reg. (EC) No 396/2005, according to Art. 18(1)(b) of that Regulation. Up till now *Bacillus thuringiensis* subsp. *aizawai* strain ABTS-1857 is not included in Annex IV due to delay at EFSA. Moreover, the default MRL of 0.01 mg/kg is not applicable because agencies are not used to follow enforcement or maintenance procedures for micro-organisms. Furthermore, the evaluation of the renewal to still going on to disprove the EFSA opinion that *Bacillus thuringiensis* subsp. *aizawai* strain Based on available information it can be concluded that the risk for consumers due to possible exposure of Bta ABTS-1857 is acceptable.
and pathogenic *B. cereus* strain are comparable.

2.7.3 Summary of residue behavior resulting

The presence of BTa ABTS-1857 in foodstuffs following its use as a plant protection product is of no risk and a consumer risk assessment is not required. Neither a residue definition nor MRLs are considered necessary. So based on available information it can be concluded that the risk for consumers due to possible exposure of Bta ABTS-1857 is acceptable.

2.8 Fate and behaviour in the environment

In the natural environment under favourable conditions *Bacillus thuringiensis* (Bt) cells exist in an active vegetative state where growth and colony formation can occur. Once conditions become unsuitable for continued growth and survival, sporulation takes place where endospores and crystalline inclusions, or proteins, are formed and the vegetative cells lyse. The endospores exist in a cryptobiotic state, akin to suspended animation and are quite durable. The crystalline proteins are the source of the δ-endotoxins which are damaging to specific insect species. When crystal proteins are ingested by insects, alkaline conditions in the gut initiate breakdown of the proteins which releases the δ-endotoxins. These immediately begin to interfere with internal cell gut structure soon leading to a cessation of feeding and, when enough crystal proteins were ingested, to eventual starvation.

2.8.1 Summary of fate and behaviour in soil

The EFSA peer review of the pesticide risk assessment of the active substance *Bacillus thuringiensis* subsp. *aizawai* highlighted that it should be demonstrated that, under the conditions of use, *Bacillus thuringiensis* subsp. *kurstaki* (Btk) crystalline proteins (δ-endotoxins) or any of their transformation products retaining insecticidal activity will not contaminate groundwater above the regulatory limit of 0.1 µg/L. The new information on persistence and multiplication in soil has been included to address this data gap.

With regards physical properties, Calabrese *et al.* (1980) used gel electrophoresis to determine the number and size of the subunits present in the protein crystals from 16 strains of *Bacillus thuringiensis*. The calculated molecular weights (MW) fell into three major categories whose crystals exhibited the following protein banding patterns: type I, high MW only (140,000-160,000 Da); type II, both high MW and medium MW (60,000 and 150,000 Da); and type III, low MW only (40,000-50,000 Da). Faust *et al.* (1974) showed the possible molecular weights of the δ-endotoxin to be 230,000 Da.

To address persistence of the crystalline protein (δ-endotoxin), various half-lives have been found in the literature, ranging from hours and days to months. Haddad *at al.* (2005) found the half-life of viable *Bacillus thuringiensis* spores in maize leaves to be 18.4, 16.5 and 13.6 hours for a half-dose, dose and double-dose of Dipel (containing Btk), respectively. Sundaram *et al.* (1996) measured the effect of two tracer dyes (Erio Acid red (EAR) and Acid Black 48 (AB-48)) on the persistence of *Bacillus thuringiensis* subsp. *kurstaki* δ-endotoxin after spraying two commercial formulations (Foray 48B and Foray 76B). The δ-endotoxin persisted on foliage only for 7 d post-spray when the EAR dye was added to Foray 48B, compared to 10 d when no dye was added. The average DT_{50} of disappearance was 17.4 h for Foray 48B with EAR and 20.9 h when no dye was added. The average DT_{50} was 27.9 h for Foray 76B with EAR and 22.2 h when no dye was added. Persistence was the longest (14 d) when the AB-48 dye was added to the Foray 76B (DT_{50} of 44.9 h). Although these half-lives are measured on foliage and not soil, this information shows that degradation of *Bacillus thuringiensis* is fairly rapid when exposed to sunlight.

West *et al.* (1984) found that there was a rapid loss of parasporal crystal insecticidal activity in natural soil, which was attributed to the presence of microbiota. The parasporal crystal was found to retain full insecticidal activity for three days and then decline. The study by West (1984) quantified the DT_{50} of the parasporal crystal in untreated soil as 2.7 days. The extent of degradation was significantly reduced by addition of soluble organic supplement (5.2-5.8 days). This study also indicated that the parasporal crystal protoxins are readily degraded by soil microorganisms.

Similar half-lives of *Bacillus thuringiensis* of 2.4-3.1 days and < 3 days have also been determined in field cotton (Ali and Young, 1993) and maize and common bean leaves (Sánchez-Yáñez and Peña-Cabriales, 2000), respectively. As well as finding that *Bacillus thuringiensis* spores had a limited persistence of less than 3 days on maize and common bean leaves, Sánchez-Yáñez and Peña-Cabriales (2000) also found that *Bacillus thuringiensis* spores were

not viable after inoculation in sterilised soil, indicating that the organic matter of the sterilised soil did not favour spore persistence. In the non-sterilised soil, competition and predation from indigenous organisms caused rapid loss of spore viability. This suggests that, at least under these experimental conditions (maize and common bean leaves and the soil tested) *Bacillus thuringiensis* spores were unable to persist.

Bai *et al.* (2007) investigated the impacts of soil water content, pH and temperature on degradation of Cry1Ab protein. Half-life values were measured in the range of 1.8 to 4.0 days. Degradation was found to be affected by soil water content, pH and temperature but effects of soil pH and temperature were relatively greater. Degradation was generally slower under lower soil pH and temperature conditions. Hung *et al.* (2016) determined the half-life of the biopesticide under natural soil conditions to be approximately one week. Feng *et al.* (2011) determined the DT_{50} of Cry1Ab protein released from 34B24 and 1246 x 1482 straw to be 0.97-9.97 d and 0.75-10.89 d, respectively. The results suggested that soil temperature had significant effects on the degradation of Cry1Ab protein, with a higher degradation rate at higher temperature, but soil water content and pH had no obvious effects on the degradation of Cry1Ab protein.

The degradation of the cry protein has been shown to be more rapid in water than soil ($t_{1/2}$ four days in water, nine days in soil) and that presence of the cry protein is fairly uncommon in aquatic environments (Douville *et al.*, 2005). In Eastern red cedar, Hostetter *et al.* (1975) found that residual activity of Dipel could still be detected 14 d post-treatment. Wang *et al.* (2007) found rapid degradation of Cry1Ab protein in paddy soils under aerobic conditions, with half-lives ranging from 19.6 to 41.3 days.

The results from the studies by Vettori *et al.* (2003) conducted in Sardinian soils, and Hendriksen and Carstensen (2013) showed that *Bacillus thuringiensis* subsp. *kurstaki* can persist for protracted periods of time in soil. The results from Vettori *et al.* (2003) indicated that *Bacillus thuringiensis* subsp. *kurstaki* and its toxin introduced into soils in sprays can persist for at least 88 months for *Bacillus thuringiensis* subsp. *kurstaki* and at least 28 months for its toxin. The results from Hendriksen and Carstensen (2013) showed that the bacterium could survive at relative low densities in 13 years after spraying. However, it is important to note that the levels of *Bacillus thuringiensis* subsp. *kurstaki* measured were below, or comparable with, typical background levels, thus posing no risk to the environment.

The results of these studies show that the persistence of the microbial pest control agent (MPCA) *Bacillus thuringiensis* and its crystalline protein (δ-endotoxin) is largely a function

of the study conditions (*i.e.* soil type, temperature, pH, moisture, photo-degradation, native microflora etc.). As such, under the majority of conditions *Bacillus thuringiensis* and its crystalline protein (δ-endotoxin) is degraded relatively quickly, however as demonstrated by Vettori *et al.* (2003) and Hendriksen and Carstensen (2013) it is not impossible for *Bacillus thuringiensis* subsp. *kurstaki* and its crystalline protein (δ-endotoxin) to persist in soils for extended periods of time, although the levels of *Bacillus thuringiensis* subsp. *kurstaki* measured did not exceed typical background levels. Since *Bacillus thuringiensis* subsp. *aizawai* and its crystalline protein (δ-endotoxin) occur naturally and ubiquitously in the environment (notifier: please include a reference), it is expected the levels reach background concentrations and densities within weeks after application. Over time (typically in the range of approximately 1-14 days) levels of *Bacillus thuringiensis* subsp. *aizawai* and will decline to numbers of bacteria found in the soil naturally.

2.8.2 Summary of fate and behaviour in water

The fate and behaviour of *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 in water was evaluated during the Annex I Inclusion. No additional studies have been performed and no further data are provided. The fate and behaviour of *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 in water is discussed in detail in the corresponding document of the EU review dossier where the references cited from the scientific literature can be found. A brief overview of this information is provided below.

Although *Bacillus thuringiensis* subsp. *kurstaki* has been shown to survive to some extent in water, viability in the natural aquatic environment is influenced by many biological, chemical and physical factors. Predation by bacteriophages, protozoans and other lower animal forms undoubtedly plays a role in controlling the bacteriological population in the aquatic environment.

Bacillus thuringiensis subsp. *kurstaki* is not regarded as an autochthonous inhabitant of aquatic environments and does not find optimal conditions for growth in the aquatic environment (*e.g.* waters are poor in organic carbon content). Therefore, proliferation is not likely to occur. Bacterial cells and especially spores may survive, but will be subject to natural competition in the diverse microbiota of natural waters. Survival of the applied ABTS-351 is not expected to cause any environmental or health impact.

2.8.3 Summary of fate and behaviour in air

The fate and behaviour of *Bacillus thuringiensis* subsp. *aizawai* strain ABTS-1857 in the atmosphere was evaluated during the Annex I Inclusion. No additional studies have been performed and no further data are provided. The fate and behaviour of *Bacillus thuringiensis* subsp. *aizawai* strain ABTS-1857 in air is discussed in detail in the corresponding document of the EU review dossier where the references cited from the scientific literature can be found. A brief overview of this information is provided below.

Following application of the XenTari® WG formulation as proposed, spray drift can occur which may result in temporary concentrations of the microbial pest control agent in the atmosphere. However, *Bacillus thuringiensis* subsp. *aizawai* is expected to undergo rapid degradation in the atmosphere since inactivation by solar radiation is a very important factor causing loss of activity and degradation of bacteria spores and δ-endotoxin crystals in the field environment. The survival and persistence of *Bacillus thuringiensis* subsp. *aizawai* in air is therefore expected to be very limited.

2.8.4 Summary of mobility

The mobility of *Bacillus thuringiensis* subsp. *aizawai* strain ABTS-1857 was evaluated during the Annex I Inclusion. No additional studies have been performed and no further data are provided. The mobility of *Bacillus thuringiensis* subsp. *aizawai* strain ABTS-1857 is discussed in detail in the corresponding document of the draft assessment report (DAR, 2008 and Addendum, 2011) where the references cited from the scientific literature can be found. A brief overview of this information is provided below.

Various experiments examining the movement of *Bacillus thuringiensis* in soils following spraying of commercial products containing *Bacillus thuringiensis* showed little or no movement. Even one year following an application onto a sandy clay loam soil in a cabbage field in Denmark, 77% of recovered *Bacillus thuringiensis* remained in the 0 to 2 cm topsoil layer. In experiments in Japan, it was found that under artificially and naturally irrigated conditions, there was no translocation of sprayed *Bacillus thuringiensis* into the soil down to a depth of 10 cm. Artificial irrigation with 450 mm simulated rainfall in a soil column showed no movement through 6 cm of volcanic ash and only a few bacteria were detected in the flow

through water from movement through a 6 cm column of alluvium sand. Under natural rainfall conditions, a reduction of *Bacillus thuringiensis* numbers of 71 to 99% in the top 0 to 1 cm of surface soil occurred in the first week of a 34-day post-application observation period. No dispersion of *Bacillus thuringiensis* was detected in the field soils below 1 cm to investigated depths of 9 to 10 cm, 19 to 20 cm and 29 to 30 cm. It can therefore be concluded that movement of *Bacillus thuringiensis* through the soil by leaching is unlikely to occur. In addition, the adsorption and binding of crystalline proteins (protoxins) and Cry toxins from *Bacillus thuringiensis* has been demonstrated to occur readily, rapidly and strongly onto the clay fraction and clay humic acid complexes of soils, with desorption occurring far less readily.

2.9 Effects on non-target species

2.9.1 Summary of effects on birds (and other terrestrial vertebrates)

The previous DAR included a risk assessment according to SANCO document 4145/2000. RMS would like to note that it was agreed in PRAPeR M2 that the guidance document SANCO/4145/2000 was intended for chemical substances and is considered less relevant for plant protection products containing micro-organisms. During PRAPeR M2 it was agreed that, with the lack of appropriate exposure scenario's for micro-organisms, a worst case risk assessment could be performed by comparing the amount of CFU applied, or present in the application liquid to the endpoint of the study.

The RMS included the following risk assessment for birds and mammals.

- **Birds**

The data of both studies provided a LD50 of > 3.4 x 10^{11} CFU/kg b.w/d.
The density of spores in the WG formulation is 5 x10^{13} CFU/kg. The maximum of product applied is 0.002 kg/L which results in a maximum concentration in the spray liquid of 1 x 10^{11} CFU/L.

Daily dose birds:
The exposure via drinking water is considered relevant. According to the EFSA bird mammal guidance
document (EFSA Journal 2009; 7(12):1438), the worst-case for drinking water is a small granivorous bird with a body weight of 15.3 g, with a drinking rate of 7.0 mL/day, equivalent to 0.46 L/kg bw/d. Based on the worst PECsw of 9.84 x 10^5 CFU/L for applications in fruiting vegetables, the daily dose is 4.5 x 10^5 CFU/kg bw/d. This value is below the endpoint for birds and therefore the risk through drinking water is considered acceptable.

- **Mammals**

The acute oral LD_{50} was greater than 5050 mg/kg bw (corresponding to 9.24 $x10^{10}$ CFU/kg b.w.). The test substance is not toxic, infective or pathogenic on the basis of the acute oral toxicity study in rats.

Daily dose mammals:
The application liquid contains 1 x 10^{11} CFU/L . The daily water intake of a small granivorous mammal with a body weight of 21.7 g is 0.24 L/kg bw/d. Considering the worst PECsw of 9.84 x 10^5 CFU/L for applications in fruiting vegetables, the daily dose is 2.36 x 10^5 CFU/kg bw/d. This value is below the endpoint for mammals and therefore the risk through drinking water is considered acceptable.

2.9.2 Summary of effects on aquatic organisms

The RMS recalculated PEDsw for eight applications in fruiting vegetables of 1.7 x 10^{13} CFU/ha. As drift values of 1.52% based on Rautmann drift values for seven (or more) applications on fruit crops and a TOXSWA standard ditch of 210 L/m^2 results in a PEDsw of 9.84 x 10^5 CFU/L. This value can be used for the ecotoxicological risk assessment.

Risk Assessment for Fish
The acute toxic endpoint for fish of 2 x 10^7 CFU/L and the chronic toxic endpoint of 1.74 x 10^{10} CFU/L are much higher than the conservative estimated PEDsw of 9.84 x 10^5 CFU/L. Therefore, *Bacillus thuringiensis* ssp. *aizawai* ABTS-1857 can be considered to pose a low risk to fish.

Risk Assessment for Aquatic Invertebrates
The acute toxic endpoint for aquatic invertebrates of 2.4 x 10^8 CFU/L and the chronic endpoint of 1.94 x 10^8 CFU/L are much higher than the conservative estimated PEDsw of 9.84 x 10^5 CFU/L. Therefore, *Bacillus thuringiensis* ssp. *aizawai* ABTS-1857 can be considered to pose a low risk to aquatic invertebrates.

Risk Assessment for Algae
The toxic endpoint for algae of 2.26 x 10^9 CFU/L is much higher than the conservative estimated PEDsw of 9.84 x 10^5 CFU/L. Therefore, *Bacillus thuringiensis* ssp. *aizawai* ABTS-1857 can be considered to pose a low risk to algae.

2.9.3 Summary of effects on bees

Risk assessment product
There were no effects on honeybee colonies at applications two times higher than the max field appli-

cation rate of 1 x10^{11} CFU/L.

No mortality to bumblebees was observed through the oral and contact exposure at rates equivalent to maximum field application rate and two times higher the field application rate, respectively. Nevertheless, the concentration in the topical test needs to be confirmed by the applicant.

In the study by Mommaerts et al. (2009), 100% mortality and as well reduction in reproduction via sugar water was observed at doses similar to the current field application rates. Similar effects can be expected for the current applications.

Risk assessment active substance

In the oral test the LD50 was set at 2 x 10^{11} CFU/L which is two times the maximum field application rate. No effects on honeybee larvae were observed at concentrations in diet higher than the current maximum field application rate. Based on these results, no risk to honey bees and honey bee larvae is expected through oral exposure to the active substance.

2.9.4 Summary of effects on arthropods other than bees

Species	**Test duration**	**Results/ Endpoint CFU/L**	**Exposure CFU/L**	**MoS**
Active substance				
Metaseiulus occidentalis (predatory mite, predator)	max 8 days	ER50 mortality: > 4.8 x 10^{12}	1 x 10^{11}	48
Tetranychus urticae (spider mite, prey)		ER50 mortality adults < 4.8 x 10^{12} ER50 survival protonymphs > 4.8 x10^{11} - < 4.8 x10^{12}		<48 > 4.8 - < 48
Trichogramma	max 10 days	ER50		> 48

Species	**Test duration**	**Results/ Endpoint CFU/L**	**Exposure CFU/L**	**MoS**
pretiosum		mortality: > 4.8 x 10^{12} CFU/L		
Ladybird beetle *Hippodamia convergens*	27 days	Dietary ER50 mortality: > 4.69x10^{11}		> 4.7
Green lacewing *Chysoperla carnea*	7 days	ER50 mortality: > 4.8 x 10^{11} while < 4.8 x 10^{12} ER50 reproduction (pupation success): > 4.8 x 10^{11}		> 4.8 - < 48
Species	**Test duration**	**Results/ Endpoint Kg product/ha**	**Exposure Kg product/ha**	**MoS**
Trichogramma cacoeciae	7 days	> 33.4	1	33.4

The active substance and the product do not have unacceptable effects on the arthropods used in the IPM such as *Metaseiulus occidentalis, Hippodamia convergens, Chrysoperla carnea, Trichogramma* spp. No effects on the adult agricultural pest *Tetranychus urticae* were observed while some effects on the protonymphs were seen at concentrations similar to the current application. Based on these results it can be concluded that the active substance and the product have no risk to non-target arthropods.

2.9.5 Summary of effects on earthworms and other soil non-target macro-organisms

No effects on earthworms of the active substance at 1000 mg a.s./kg dry soil were observed in a 30

day study. This concentration is above the PECsoil of 57.6 mg a.s./kg soil assuming no considerations for degradation. The risk to earthworms is therefore considered acceptable.

2.9.6 Summary of effects on soil micro-organisms

No studies are available to assess the effects of XenTari® WG or BTa ABTS-1857 on soil micro-organisms. However, there is published information that assesses the impact of various other *B. thuringiensis* subspecies. Given the nature of the host specificity of the different subspecies (between different insect groups) and the lack of taxonomic relatedness between these susceptible species and soil micro-organisms, this information is considered relevant here.

EFSA (2013) concluded that *"Bacillus thuringiensis* occurs naturally and ubiquitously in the environment. It is a common component of the soil micro-flora and has been isolated from most terrestrial habitats. Although originally recovered mainly from insects, recent studies have indicated that *B. thuringiensis* is distributed in soil sparsely but frequently and its distribution is widespread, both locally and worldwide. *B. thuringiensis* is not adapted to survive as an active member of the soil microbial community and the low potential for spore germination, growth and resporulation restricts population growth. Although specific studies were not produced by the Applicant, it can be suggested that there will be low impact on soil micro-organism populations and processes when XenTari® WG is applied according to the proposed GAP, and intended uses".

2.9.7 Summary of effects on other non target (flora and fauna)

No studies submitted. Due to the mode of action, not expected to cause any effects on terrestrial plants.

2.9.8 Summary of effects on biological methods for sewage treatment

One study submitted, however not considered relevant. Nevertheless, it is not expected that Bta will survive in the sewage treatment plant.

2.9.9 Summary of product exposure and risk assessment

Please refer to the above sections.

2.10 Classification and labelling

2.10.1 Classification and Labelling of the active substance

No classification required. As a precautionary measure the sentence “This product does contain micro-organisms and may cause sensitisation by skin contact" should be included on the label.

2.10.2 Classification and Labelling of the plant protection product

Classification and labelling for eye irritation with ‘Warning, H319' is required.. As a precautionary measure the sentence “This product does contain micro-organisms and may cause sensitisation by skin contact" should be included on the label.

2.11 Relevance of metabolites in groundwater

Not relevant for microorganisms

2.12 Consideration of isomeric composition in the risk assessment

Not relevant for microorganisms.

2.12.1 Definition of residues for monitoring

No residue definition is required.

Level 3

Bacillus thuringiensis ssp. aizawai strain ABTS-1857

3 Proposed decision with respect to the application

3.1 Background to the proposed decision

3.1.1 Proposal on acceptability against the decision making criteria – Article 4 and annex II of regulation (EC) No 1107/2009

3.1.1.1 Article 4				
		Yes	No	
i)	It is considered that Article 4 of Regulation (EC) No 1107/2009 is complied with. Specifically the RMS considers that authorisation in at least one Member State is expected to be possible for at least one plant protection product containing the active substance for at least one of the representative uses.			*Inconclusive; depends on the outcome of the data gaps*
3.1.1.2 Submission of further information				
		Yes	No	
i)	It is considered that a complete dossier has been submitted	x		
ii)	It is considered that in the absence of a full dossier the active substance may be approved even though certain information is still to be submitted because: (a) the data requirements have been amended or refined after the submission of the dossier; or (b) the information is considered to be confirmatory in nature, as required to increase confidence in the decision.			*n.a.*
3.1.1.3 Restrictions on approval				
		Yes	No	
	It is considered that in line with Article 6 of Regulation (EC) No 1107/2009 approval should be subject to conditions and restrictions.		x	

3.1.1.4 Criteria for the approval of an active substance				
Dossier				
		Yes	No	
	It is considered the dossier contains the information needed to establish, where relevant, Acceptable Daily Intake (ADI), Acceptable Operator Exposure Level (AOEL) and Acute Reference Dose (ARfD).	X		No reference values are required.
	It is considered that the dossier contains the information necessary to carry out a risk assessment and for enforcement purposes (relevant for substances for which one or more representative uses includes use on feed or food crops or leads indirectly to residues in food or feed). In particular it is considered that the dossier: (a) permits any residue of concern to be defined; (b) reliably predicts the residues in food and feed, including succeeding crops (c) reliably predicts, where relevant, the corresponding residue level reflecting the effects of processing and/or mixing; (d) permits a maximum residue level to be defined and to be determined by appropriate methods in general use for the commodity and, where appropriate, for products of animal origin where the commodity or parts of it is fed to animals; (e) permits, where relevant, concentration or dilution factors due to processing and/or mixing to be defined.	X		No consumer risk assessment is required (see Volume 3 MA B.7)
	It is considered that the dossier submitted is sufficient to permit, where relevant, an estimate of the fate and distribution of the active substance in the environment, and its impact on non-target species.	x		
Efficacy				
		Yes	No	
	It is considered that it has been established for one or more representative uses that the plant protection product, consequent on application consistent with good plant protection practice and having regard to realistic conditions of use is sufficiently effective.	x		

Relevance of metabolites				
		Yes	No	
	It is considered that the documentation submitted is sufficient to permit the establishment of the toxicological, ecotoxicological or environmental relevance of metabolites.	X		The metabolites are not considered to be relevant.
Composition				
		Yes	No	
	It is considered that the specification defines the minimum degree of purity, the identity and maximum content of impurities and, where relevant, of isomers/diastereo-isomers and additives, and the content of impurities of toxicological, ecotoxicological or environmental concern within acceptable limits.	X		Not a relevant question for micro-organisms. The activity in CFU/g is provided and is relevant for a micro-organism.
	It is considered that the specification is in compliance with the relevant Food and Agriculture Organisation specification, where such specification exists.			Not relevant for microorganisms.
	It is considered for reasons of protection of human or animal health or the environment, stricter specifications than that provided for by the FAO specification should be adopted			Not relevant for microorganisms.
Methods of analysis				
		Yes	No	
	It is considered that the methods of analysis of the active substance, safener or synergist as manufactured and of determination of impurities of toxicological, ecotoxicological or environmental concern or which are present in quantities greater than 1 g/kg in the active substance, safener or synergist as manufactured, have been validated and shown to be sufficiently specific, correctly calibrated, accurate and precise.	X		Sufficient validated methods are available.
	It is considered that the methods of residue analysis for the active substance and relevant metabolites in plant, animal and environmental matrices and drinking water, as appropriate, shall have been validated and shown to be sufficiently sensitive with respect to the levels of concern.			No residue methods of analysis are required.
	It is confirmed that the evaluation has been carried out in accordance with the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) of Regulation 1107/2009.	X		

Impact on human health				
Impact on human health - ADI, AOEL, ARfD				
		Yes	No	
	It is confirmed that (where relevant) an ADI, AOEL and ARfD can be established with an appropriate safety margin of at least 100 taking into account the type and severity of effects and the vulnerability of specific groups of the population.	X		No reference values are required.
Impact on human health - proposed genotoxicity classification				
		Yes	No	
	It is considered that, on the basis of assessment of higher tier genotoxicity testing carried out in accordance with the data requirements and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as mutagen category 1A or 1B.**			Not relevant for microorganisms
Impact on human health - proposed carcinogenicity classification				
		Yes	No	
i)	It is considered that, on the basis of assessment of the carcinogenicity testing carried out in accordance with the data requirements for the active substances, safener or synergist and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as carcinogen category 1A or 1B.**			Not relevant for microorganisms
ii)	Linked to above classification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			n.a.
Impact on human health – proposed reproductive toxicity classification				

		Yes	No	
i)	It is considered that, on the basis of assessment of the reproductive toxicity testing carried out in accordance with the data requirements for the active substances, safeners or synergists and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as toxic for reproduction category 1A or 1B.**			Not relevant for microorganisms
ii)	Linked to above classification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			N.a.
Impact on human health - proposed endocrine disrupting properties classification				
		Yes	No	
i)	It is considered that **the substance SHOULD BE classified or proposed for classification** in accordance with the provisions of Regulation (EC) No 1272/2008, as **carcinogenic category 2 and toxic for reproduction category 2 and on that basis shall be considered to have endocrine disrupting properties**			Not relevant for microorganisms
ii)	It is considered that **the substance SHOULD BE classified or proposed for classification** in accordance with the provisions of Regulation (EC) No 1272/2008, as **toxic for reproduction category 2 and** in addition the RMS considers the substance **has toxic effects on the endocrine organs and on that basis shall be considered to have endocrine disrupting properties**			Not relevant for microorganisms
iii)	Linked to either i) or ii) immediately above. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact			n.a.

	with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			
Fate and behaviour in the environment				
Persistent organic pollutant (POP)				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a persistent organic pollutant (POP) as laid out in Regulation 1107/2009 Annex II Section 3.7.1.		X	Micro-organisms are not considered persistent. Bt are naturally present in soil.
Persistent, bioaccumulative and toxic substance (PBT)				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a persistent, bioaccumulative and toxic (PBT) substance as laid out in Regulation 1107/2009 Annex II Section 3.7.2.		X	Micro-organisms are not considered persistent. Bt are naturally present in soil.
Very persistent and very bioaccumulative substance (vPvB)				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a a very persistent and very bioaccumulative substance (vPvB) as laid out in Regulation 1107/2009 Annex II Section 3.7.3.		X	Micro-organisms are not considered persistent. Bt are naturally present in soil.
Ecotoxicology				
		Yes	No	
	It is considered that the risk assessment demonstrates risks to be acceptable in accordance with the criteria laid down in the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) under realistic proposed conditions of use of a plant protection product containing the active substance, safener or synergist. The RMS is content that the assessment takes into account the severity of effects, the uncertainty of the data, and the number of organism groups which the active substance, safener or synergist is expected to affect adversely by the intended use.			Bees <u>Risk assessment product</u> There were no effects on honeybee colonies at applications two times higher than the max field application rate of 1 x10^{11} CFU/L. No mortality to bumblebees was observed through the oral and contact exposure at rates equivalent to maximum field application rate and two times higher the field application rate, respectively. Nevertheless, the concentration in the topical test needs to be confirmed by

				the applicant. In the study by Mommaerts et al. (2009), 100% mortality and as well 100% reduction in reproduction via sugar water was observed at 1.5 x 10^4IU/mL a dose similar to the current field application rates. The current range of application is 7.5 x 10^3 IU/mL to 3 x 10^4IU/mL . Therefore similar effects can be expected for the current applications. The RMS is of opinion that if no further information is provided a restriction sentence and a warning sentence are necessary prohibiting the application of the product when the crop is flowering Further infectivity and pathogenicity must be addressed. The possible effects of Cry toxins on bees and bumblebees must be addressed
	It is considered that, on the basis of the assessment of Community or internationally agreed test guidelines, the substance **HAS** endocrine disrupting properties that may cause adverse effects on non-target organisms.		X	Not relevant for a micro-organism
	Linked to the consideration of the endocrine properties immediately above. It is considered that the exposure of non-target organisms to the active substance in a plant protection product under realistic proposed conditions of use is negligible.	X		Endocrine disruption is not relevant for a micro-organism
	It is considered that it is established following an appropriate risk assessment on the basis of Community or internationally agreed test guidelines, that the use under the proposed conditions of use of plant protection products containing this active substance, safener or synergist: — will result in a negligible exposure of honeybees, or — has no unacceptable acute or chronic effects on colony survival and development, taking into account effects on honeybee larvae and honeybee behaviour.			Bees Risk assessment product There were no effects on honeybee colonies at applications two times higher than the max field application rate of 1 x10^{11} CFU/L. No mortality to bumblebees was observed through the oral and contact exposure at rates equivalent to maximum field application rate

				and two times higher the field application rate, respectively. Nevertheless, the concentration in the topical test needs to be confirmed by the applicant. In the study by Mommaerts et al. (2009), 100% mortality and as well 100% reduction in reproduction via sugar water was observed at 1.5 x 10^4IU/mL a dose similar to the current field application rates. The current range of application is 7.5 x 10^3 IU/mL to 3 x 10^4IU/mL . Therefore similar effects can be expected for the current applications . The RMS is of opinion that if no further information is provided a restriction sentence and a warning sentence are necessary prohibiting the application of the product when the crop is flowering Further infectivity and pathogenicity must be addressed. The possible effects of Cry toxins on bees and bumblebees must be addressed
Residue definition				
		Yes	No	
	It is considered that, where relevant, a residue definition can be established for the purposes of risk assessment and for enforcement purposes.			No residue definition is required.
Fate and behaviour concerning groundwater				
		Yes	No	
	It is considered that it has been established for one or more representative uses, that consequently after application of the plant protection product consistent with realistic conditions on use, the predicted concentration of the active substance or of metabolites, degradation or reaction products in groundwater complies with the respective criteria of the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) of Regulation 1107/2009.	X		Bt and spores are not considered mobile in soil. Leaching is not expected.

3.1.2 Proposal – Candidate for substitution

Candidate for substitution				
		Yes	No	
	It is considered that the active substance shall be approved as a candidate for substitution		x	

3.1.3 Proposal – Low risk active substance

Low-risk active substances				
		Yes	No	
	It is considered that the active substance **shall be considered of low risk.** An active substance which is a micro-organism may be considered as being of low-risk unless at strain level it has demonstrated multiple resistance to anti-microbials used in human or veterinary medicine.	X		Strain ABTS-1857 was sensitive to gentamicin, kanamycin, erthyromycin, clindamycin, vancomycin, chloramphenicol and trimethoprim/sulfamethoxazole but not sensitive to penicillin, ampicillin or cephalothin. The susceptibility was determined using standard methodology (SOP 047T-12-043A; National Committee for Clinical Laboratory Standard, 1984). Staphylococcus aureus was also included in the test to verify the test procedure (Smith, 1990).

3.1.4 List of studies to be generated, still ongoing or available but not peer reviewed

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going	Study on-going and anticipated date of completion	Study available but not peer-reviewed
3.1.4.1 Identity of the active substance or formulation				
Applicant please submit : • Summary of methods and precautions concerning handling, storage, transport or fire • Summary of procedures for destruction or decontamination • Summary of emergency measures in case of an accident • Packaging and compatibility of the preparation with proposed packaging materials • Effectiveness of the cleaning procedures	Relevant information	Not relevant	Not relevant	Not relevant
Ad C.1.1.2: Applicant to provide a statement that the production process is still the same as described in the DAR.	Relevant information	Not relevant	Not relevant	Not relevant
Ad C.1.2.2: Applicant please provide a statement whether the MPCA or the product XenTari WG was analysed. The given names are confusing. Moreover, please add for the microbial contaminants the infor-	Relevant information	Not relevant	Not relevant	Not relevant

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going	Study on-going and anticipated date of completion	Study available but not peer-reviewed
mation how many grams were taken for analysis.				
Ad C.1.2.3: Applicant please provide the production dates of the analysed batches batches and provide information whether the MPCA is analysed for spore counts of Bacillus thuringiensis aizawai ABTS-1857.	Relevant information	Not relevant	Not relevant	Not relevant
Ad C.1.1.1: Applicant please confirm or provide the proper address for the manufacturing plant for the active m.o. In Benzon, 2016 (house-fly test of technical for presence/absence of beta-exotoxin), the manufacturing plant of the tested batches is indicated as: 2142 350th Street Osage, Iowa.	Relevant information	Not relevant	Not relevant	Not relevant
Ad B.2.1.2 MA: Please address the point B.2.1.2 "Origin and natural occurrence" with more recent literature about the background.	Relevant information	Not relevant	Not relevant	Not relevant
Af. B.2.1.2 MA: Applicant please add the information on Anon. (1995) and Smith (1990) in the reference list.	Relevant information	Not relevant	Not relevant	Not relevant
Ad B.2.2.1 MA: Applicant please submit the references used to describe the target organism(s).	Relevant information	Not relevant	Not relevant	Not relevant
Ad B.2.2.2 MA: Applicant please address also the further potential insecticidal proteins as e.g. beta exotoxin and the secreted *vip* toxins (see Palma et al 2014; BACILLUS	Relevant information	Not relevant	Not relevant	Not relevant

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going	Study on-going and anticipated date of completion	Study available but not peer-reviewed
THURINGIENSIS TOXINS: AN OVER-VIEW OF THEIR BIOCIDAL ACTIVITY Toxins, 6, 3296-3325.				
Ad B.2.4 MA: The text explained that Bacillus spores are resistant to desiccation, heat, ultraviolet irradiation and other environmental factors such as chemical disinfectants. This statement should be proven with studies or relevant papers, because there are indications that Bta biopesticide spores are rather sensitive to UV light. Applicant explain more about the UV sensitivity whether the statement is about the spore or the vegetative stage and include more detailed information. Furthermore include more information observed in the study of Benoit.	Relevant information	Not relevant	Not relevant	Not relevant
Ad B.2.4 MA: The information provided is scarce and basically ignores the data requirements. According to Commission Regulation (EU) No 283/2013 (Annex Part B Micro-organisms including viruses) four points need to be addressed in chapter B.2.4, i.e.: • Information on the life cycle of the micro-organism, described symbiosis, parasitism, competitors, predators, etc., including host organisms, as well as vectors for viruses, must be presented. • The generation time and the type of	Relevant information	Not relevant	Not relevant	Not relevant

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going	Study on-going and anticipated date of completion	Study available but not peer-reviewed
reproduction of the micro-organism must be stated. • Information on the occurrence of resting stages and their survival time, their virulence and infection potential must be provided. • The potential of the micro-organism to produce metabolites, including toxins that are of concern for human health and/or the environment, in its different development stages after the release, must be indicated. Furthermore, no references have been provided. A literature search including specific search terms like development, life cycle, replication, proliferation, survival, virulence, infection, metabolite, toxin or alike is missing.				
Ad B.2.4 MA: Applicant please describe the result of the degradation over time measurements of spores and parasporal crystals in soil.	Relevant information	Not relevant	Not relevant	Not relevant
Ad B.2.5 MA: Applicant please add the references used in the alinea about survival and viability in the soil, natural aquatic environment and atmosphere Please also add the question on results of degradation over time in soil.	Relevant information	Not relevant	Not relevant	Not relevant

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going	Study on-going and anticipated date of completion	Study available but not peer-reviewed
Ad B.2.5 MA: Applicant please add the reference used to explain inactivation by solar radiation of Bacillus thuringiensis bacteria spores and crystal proteins	Relevant information	Not relevant	Not relevant	Not relevant
Ad B.2.5 MA: if new publications have been found relevant for the RAR they should be cited within context. Simply providing the summary or an abstract is not sufficient. EFSA (2016) has not been included in the References relied on chapter. As regards the paper by Ruan et al. (2015), the notifier claimed that it resulted from the literature search. However, in Table 4 and Table 6 presented in section B.6 MA this paper is neither listed as relevant nor as excluded. Besides, this paper is a response of the authors to a response of Loguercio and Argolo-Filho (2015) to the original paper by Ruan et al. (2015). Therefore, it is presented ignoring the context of its reason, which is scientifically not sound.	Relevant information	Not relevant	Not relevant	Not relevant
Ad B.2.6 MA: The information provided by the notifier is scarce. No reference has been provided concerning the formally recognised members of the Bacillus cereus group. The following citations have not been included in the References relied on chapter:	Relevant information	Not relevant	Not relevant	Not relevant

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going	**Study on-going and anticipated date of completion**	**Study available but not peer-reviewed**
Raymond and Federici (2017), Rosenquist et al. (2005), Frederiksen et al. (2006), Guinebretière et al. (2010), Ray-mond et al. (2010b), Raymond and Bonsall (2013), Hernandez et al. (2000), Ramisse and Ducoureau (1998).				
Ad B.2.7 MA: Three references have been listed that have not been referred to (cited) in the main text, i.e. Ferreira et al., (2003); van der Auwera et al., 2007, and Short et al., (2015). Applicant please address.	Relevant information	Not relevant	Not relevant	Not relevant
Ad B.2.8 MA: Commercial products containing Bacillus thuringiensis subsp. aizawai, strain ABTS-1857 have been shown not to contain β-exotoxins or an high amount of enterotoxins. Moreover, Bta strain ABTS-1857 produces the Cry toxins (Cry1Aa, Cry1Ab, Cry 1C, and Cry 1D). β-exotoxin and the emetic toxin cereulide are not present in manufacturing of Bta strain ABTS-1857. Applicant please provide all references to address the presence or absence of the mentioned metabolites (see also B.6.1.1).	Relevant information	Not relevant	Not relevant	Not relevant
Ad B.2.8 MA: Applicant please address the conclusions of the studies performed by Pardo-Lopez et al (2012; KMA 2.8/03) and Palma et al. (2014; KMA 2.8/04).	Relevant information	Not relevant	Not relevant	Not relevant

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going	**Study on-going and anticipated date of completion**	**Study available but not peer-reviewed**
Ad B.2.8 MA: The applicant is requested to incorporate some information from the two references de la Vega, L. M. et al. (2006) and Hsieh, F-C. et al. (2008) in the general text (at the moment there is no reference in the text to these papers). Moreover the applicant is requested to also provide summaries, similar to above, for the remaining 7 references mentioned in the text (2.8/03 to 2.8/09). The applicant is requested to explain why a lot of references used are not found in the literature search. Also Harri-am (2015) has not been included in the References relied on chapter.	Relevant information	Not relevant	Not relevant	Not relevant
Ad 2.9: The description of the study should be substantiated with more details. Applicant please insert a justification regarding the determination of bacterial growth. Which method of quantification has been used to distinguish between clear medium and turbid one?	Relevant information	Not relevant	Not relevant	Not relevant
3.1.4.2 Physical and chemical properties of the active substance and physical, chemical and technical properties of the formulation				
B.2.6/02: Bulk density (pour and tap) of powder or granules. The study was accepted in the original DAR, but the pour and tap density are not reported and are required according 284/2013 and should therefore be	Relevant information	Not relevant	Not relevant	Not relevant

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going	Study on-going and anticipated date of completion	Study available but not peer-reviewed
provided.				
3.1.4.3 Data on uses and efficacy				
No data gap				
3.1.4.4 Data on handling, storage, transport, packaging and labelling				
No data gap				
3.1.4.5 Methods of analysis				
Ad B.5.1.2 MA: van der Vossen et al (2008) used comprehensive DNA and RNA analyses to characterize genetic diversity and gene expression in a genome-wide manner. Applicant please clarify whether this method can also be used for Bacillus thuringiensis ssp. aizawai (ABTS 1857).	Relevant information	Not relevant	Not relevant	Not relevant
Ad B.5.1.2 MA: Applicant please clarify the summary of KMA 1.3/03 Anon. (2015) (a) & (b) with more text and evidence (references) and also take into account the sentence presented in B.6: The genetic sequence of Bacillus thuringiensis ssp. aizawai (strain ABTS 1857) presented in Document J shows the absence of some (which?) enterotoxin genes and also shows the absence of an operon including the gene for a principal enterotoxin (which?). Scientific Evidence (not a power	Relevant information	Not relevant	Not relevant	Not relevant

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going	Study on-going and anticipated date of completion	Study available but not peer-reviewed
point)???				
3.1.4.6 Toxicology and metabolism				
Ad. B.6 MA: RMS general remark: all references, including those in the footnotes, should be provided, accompanied by a notifier's evaluation and summary. Only for some general references/reports such as the EFSA BIOHAZ opinion on Bacillus cereus, the internet link will be sufficient (i.e. no notifier's evaluation and summary necessary). When text on study evaluations are copied from e.g. the EFSA BIOHAZ opinion, without further detailed assessment of that individual study, this should be clearly indicated.	Relevant information	Not relevant	Not relevant	Not relevant
Ad B.6.1.1 MA: A large number of Cry proteins have been identified; cry genes are located on large plasmids. *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS-1857) produces the Cry toxins Cry1Aa, Cry1Ab, Cry 1C, and Cry 1D. Applicant please provide the reference for this (see also B.1.3.3).	Relevant information	Not relevant	Not relevant	Not relevant
Ad. B.6.1.1MA: It is unclear, however, whether preformed *B. cereus* enterotoxins can survive conditions (low pH, presence of protease enzymes) in the mammalian gastrointestinal tract or whether symptoms are caused by de novo production in the gastrointestinal tract . (summary?) Other work by the	Relevant information	Not relevant	Not relevant	Not relevant

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going	Study on-going and anticipated date of completion	Study available but not peer-reviewed
same authors (summary?) also suggests that vegetative cells (but not spores) of B. cereus are deactivated by conditions in the mammalian gastrointestinal tract, and that food poisoning may be due to the presence of spores rather than to vegetative cells. Applicant please submit evidence.				
Ad B.6.1.1 MA: The majority of *B. thuringiensis* strains investigated have been shown not to produce enterotoxins. Applicant please provide the reference for this.	Relevant information	Not relevant	Not relevant	Not relevant
Ad B.6.1.1 MA: *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857) has been shown not to produce β-exotoxin, cytolytic proteins or enterotoxins during the production process (reference? And cytotoxin K? And why only talk about "during the production process"? How about after the production process, eg after ingestion of the m.o. by humans? Damgaard shows that ABTS 1857 has the genes to produce enterotoxins! Applicant please explain and give enough evidence. Applicant please submit the report describing the comparative analysis of the genetics of enterotoxins.	Relevant information	Not relevant	Not relevant	Not relevant
Ad B.6.1.1 MA: The genetic sequence of *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857) presented in volume 4 shows	Relevant information	Not relevant	Not relevant	Not relevant

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going	Study on-going and anticipated date of completion	Study available but not peer-reviewed
the absence of some (which?) enterotoxin genes and also shows the absence of an operon including the gene for a principal enterotoxin (which?). Applicant please explain and give enough evidence (not a power point).				
Ad B.6.1.1 MA: Genetic analysis of *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857) presented in Document J of this dossier confirms the absence of the emetic toxin gene from the genome of this particular strain (reference?).	Relevant information	Not relevant	Not relevant	Not relevant
Ad B.6.1.1 MA: Some *B. thuringiensis* strains do produce enterotoxins; however studies with *B. cereus* have shown that preformed enterotoxins are destroyed by cooking and by the low pH and enzymes present in the mammalian gastrointestinal tract. Applicant please provide the reference for this.	Relevant information	Not relevant	Not relevant	Not relevant
Ad B.6.1.1 MA: Commercial *Bt* strains have been demonstrated to grow significantly slower than pathogenic (or even non-pathogenic) strains of *B. cereus*. Applicant please provide the reference for this.	Relevant information	Not relevant	Not relevant	Not relevant
Ad B.6.1.1 MA: Bacterial enterotoxins are shown not to specifically bind to mammalian intestinal cells (reference?) and do not affect their function. Applicant please provide the	Relevant information	Not relevant	Not relevant	Not relevant

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going	Study on-going and anticipated date of completion	Study available but not peer-reviewed
reference for this or please complete the new added paragraph. It is not written very well and be sure all the new references are submitted.				
Ad B.6.1.1 MA; They (commercial Bt strains, including *Bta* ABTS 1857) do not persist on treated crops until the moment of consumption of the harvested good at relevant levels (germination and growth in either environment apart from host insect guts is rather unlikely). Applicant please provide the reference for this.	Relevant information	Not relevant	Not relevant	Not relevant
Ad B.6.1.1 MA study Berlitz DL (2006): The entire article, except for the summary, indicates that (Wistar) mice are used. Since there is no mice strain called Wistar, it is assumed that the authors used Wistar rats for their experiment. Applicant please address.	Relevant information	Not relevant	Not relevant	Not relevant
Ad B.6.1.1 MA: Studies specifically carried out to study the effects of Btk strain ABTS-351on gut microflora and to see if the *Btk* strain ABTS-351 would germinate in a mock gut, have shown no adverse effects upon the microflora or germination in the gut media. Applicant please provide the reference for this.	Relevant information	Not relevant	Not relevant	Not relevant
Applicant please check and address the studies and fill in the references in the general	Relevant information	Not relevant	Not relevant	Not relevant

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going	Study on-going and anticipated date of completion	Study available but not peer-reviewed
text in B.6.1.1.3.				
Ad B.6.1.1 MA: Phage typing confirmed all the clinical Bacillus isolates to be type 2. Applicant please explain.	Relevant information	Not relevant	Not relevant	Not relevant
Ad. B.6.1.1. MA: Could applicant explain why the study performed by Mezzomo 2015 was withdrawn?	Relevant information	Not relevant	Not relevant	Not relevant
Ad B.6.1.1 MA: Based on data on the very low levels of diarrhoeal exotoxin (?? Which toxin is this? Please be consistent in naming the toxins) production reported for *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857). Applicant please provide the reference for this.	Relevant information	Not relevant	Not relevant	Not relevant
Ad literature search (see B.6 MA): 3 articles 1) Freire IS, Miranda-Vilela AL, Barbosa LCP, Martins ES, Monnerat RG & Grisolia CK (2014a) (Toxins 6: 2872-2885), 2) Mezzomo BP, Miranda -Vilela AL, de Souza Freire I, Barbosa LCP, Portilho FA & Grisolia CK (2015) (Journal of Hematology & Thromboembolic Diseases 1(1)) and 3) Mezzomo BP, Miranda -Vilela AL, Barbosa LCP, Albernaz VL & Grisolia CK (2015) (Environmental Toxicology 31(8):970-978) are used and cannot be found in the literature	Relevant information	Not relevant	Not relevant	Not relevant

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going	Study on-going and anticipated date of completion	Study available but not peer-reviewed
search. See also for references used in B.2.6 and B.2.7 and may be in other chapters. Applicant please explain.				
1) RMS did not find a literature search for metabolites only or in combination with Bta. Therefore, applicant please perform a correct literature search on metabolites only or in combination with Bta based on the EFSA guidance. Furthermore, the report of studies excluded from the risk assessment after detailed assessment of full-text documents contains often the wordings "Not determined to be relevant. Applicant please explain with more text why this is not relevant. 2) The search terms presented in chapter B.6.4, Table 2, (p. 57) are mainly not representative for section B.2. While the terms growth, spore coat, hydrophobicity, adherence, and food poisoning may not be inappropriate per se, they do not reflect the general data requirements laid down by Commission Regulation (EU) No 283/2013 (Annex Part B Micro-organisms including viruses) for section B.2. Other search terms defined for the toxicology, environmental fate, and ecotoxicology sections like infect?, pathogen?, enviro?, and repro? are also appropriate for section B.2 but not sufficient to fully	Relevant information	Not relevant	Not relevant	Not relevant

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going	**Study on-going and anticipated date of completion**	**Study available but not peer-reviewed**
address the data requirements for this section. We agree with RMS that a specific search concerning metabolites is missing. Apart from that, we think that a more specific search should be performed including several more relevant terms concerning the data requirements needed for section B.2. 3) It is unclear whether the literature search was performed using a title, abstract, keyword or full text search or any other search kind. This should, however, be indicated in Table 2, (p. 57). 4) No relevance criteria have been defined in regard to OECD code IIM 2 (it should generally be noted that the relevance criteria presented in chapter B.6.4, Table 1, (p. 56) refer to OECD codes defined for chemical active substances, e.g. IIA 7 and IIA 8 instead of IIM 7, IIM 8, and IIM 9).				
Ad B.6.2.1 WG: Applicant please explain why the study performed by Durando 2011 has been performed.	Relevant information	Not relevant	Not relevant	Not relevant
3.1.4.7 Residue data				
Ad. B.7 MA: RMS general remark: all references, including those in the footnotes,	Relevant information	Not relevant	Not relevant	Not relevant

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going	Study on-going and anticipated date of completion	Study available but not peer-reviewed
should be provided, accompanied by a notifier's evaluation and summary. Only for some general references/reports such as the EFSA BIOHAZ opinion on Bacillus cereus, the internet link will be sufficient (i.e. no notifier's evaluation and summary necessary). When text on study evaluations are copied from e.g. the EFSA BIOHAZ opinion, without further detailed assessment of that individual study, this should be clearly indicated				
Ad B.7.1 MA: Germination of spores occurs only if conditions are appropriate, which is only the case after ingestion by insects or earthworms, or in the rhizosphere of several, but not all plants. Applicant please provide the reference for this.	Relevant information	Not relevant	Not relevant	Not relevant
Ad B.7.2: Treated pepper samples were stored up to 7 months, requiring a storage stability discussion. In public literature a decrease of efficacy for *B. thuringiensis* formulations was reported after freezer storage, suggesting a significant rate of inactivation for the spores. Reference: Mario Boisvert, Jacques Boisvert, 2001, Storage Stability of Two Liquid Formulations of Bacillus thuringiensis subsp. israelensis and Effect of Freezing Over Time, Biocontrol Science and Technology, 11:4,	Relevant information	Not relevant	Not relevant	Not relevant

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going	Study on-going and anticipated date of completion	Study available but not peer-reviewed
475-482, 10.1080/0958315012006750 8. Applicant please address whether either the study significantly underestimates the CFU in fresh samples or it is completely invalid. The impact on the overall conclusion in view of the intended uses could be high.				
3.1.4.8 Environmental fate and behaviour				
Since *Bacillus thuringiensis* subsp. aizawai and its crystalline protein (δ-endotoxin) occur naturally and ubiquitously in the environment (please include a reference; see summary of fate and behaviour in soil; vol 1, 2.8.1). If reference is to be made to Maeda et al. 2000 Curr Microbiol 40:418 and Armengol et al (2006) Journal of Applied Microbiology, please provide these studies and summaries).	Relevant information	Not relevant	Not relevant	Not relevant
No data on the potential interference with the detection of the other organisms are provided by the notifier. Therefore the effects on drinking water analysis is not addressed properly by the notifier. In addition, the notifier is asked if the data shown in this section can be made publically available since this is taken from the confidential part.	Relevant information	Not relevant	Not relevant	Not relevant
The RMS has requested the notifier to update the literature search and include the relevant and reliable literature on metabolites and	Relevant information	Not relevant	Not relevant	Not relevant

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going	**Study on-going and anticipated date of completion**	**Study available but not peer-reviewed**
extend the search with other terms.				
Bacillus thuringiensis spores have been found occurring naturally in marine sediments (Japan, Maeda *et al.*, 2000), dam sediments and waste collection ponds (Jordan, Obeidat, 2008). Please provide these reference and include the summaries in the MA part of the risk evaluation with typical PED of the spores.	Relevant information	Not relevant	Not relevant	Not relevant
Literature values for Koc can be found for Cry proteins. The notifier is asked to include these reference in the MA part and use these values for the proteins and recalculate the PECsw for the Cry proteins with an interval of 6 days	Relevant information	Not relevant	Not relevant	Not relevant
Please provide updated PECsw/sed calculations with a minimum interval of 6 days.	Relevant information	Not relevant	Not relevant	Not relevant
3.1.4.9 Ecotoxicology				
Reference 10.3.1/02, study by Bolckmans K. (1995), please clarify the exposure in the topical and inhalation toxicity tests (inconsistency between tables and description in the study report).	Relevant information	Not relevant	Not relevant	Not relevant
In the study by Mommaerts et al. (2009), 100% mortality and as well reduction in reproduction via sugar water was observed at doses similar to the current field application	Relevant information	Not relevant	Not relevant	Not relevant

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going	Study on-going and anticipated date of completion	Study available but not peer-reviewed
rates. Similar effects can be expected for the current applications. The applicant is requested to address this point. Further infectivity and pathogenicity must be addressed.				
Section B 9.6 of the product dossier – a weight of evidence was included in order to justify why no studies on effects on non-target soil organisms are required. The applicant is requested to add references in the text.	Relevant information	Not relevant	Not relevant	Not relevant
Please address the impact of the Cry toxins on bees and NTAs.	Relevant information	Not relevant	Not relevant	Not relevant
Reference 8.5/03, study by Sindermann et al., 2006 – the applicant is requested to specify the amount of CFU found in the test item.	Relevant information	Not relevant	Not relevant	Not relevant
Literature search – the RMS is of opinion that the Cry toxins and at least insects (i.e. non-target arthropods, bees and bumblebees, should have been included in the search terms. The applicant is requested to address this point.	Relevant information	Not relevant	Not relevant	Not relevant

3.1.5 Issues that could not be finalised

An issue is listed as an issue that could not be finalised where there is not enough information available to perform an assessment, even at the lowest tier level, for the representative uses in line with the Uniform Principles, as laid out in Commission Regulation (EU) No 546/2011, and where the issue is of such importance that it could, when finalised, become a concern (which would also be listed as a critical area of concern if it is of relevance to all representative uses).

Area of the risk assessment that could not be finalised on the basis of the available data	**Relevance in relation to representative use(s)**
Risk assessment bees and bumblebees	Outdoor and indoor uses

3.1.6 Critical areas of concern

An issue is listed as a critical area of concern:
(a) where the substance does not satisfy the criteria set out in points 3.6.3, 3.6.4, 3.6.5 or 3.8.2 of Annex II of Regulation (EC) No 1107/2009 and the applicant has not provided detailed evidence that the active substance is necessary to control a serious danger to plant health which cannot be contained by other available means including non-chemical methods, taking into account risk mitigation measures to ensure that exposure of humans and the environment is minimised, or
(b) where there is enough information available to perform an assessment for the representative uses in line with the Uniform Principles, as laid out in Commission Regulation (EU) 546/2011, and where this assessment does not permit to conclude that for at least one of the representative uses it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater or any unacceptable influence on the environment.

An issue is also listed as a critical area of concern where the assessment at a higher tier level could not be finalised due to a lack of information, and where the assessment performed at the lower tier level does not permit to conclude that for at least one of the representative uses it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater or any unacceptable influence on the environment.

Critical area of concern identified	**Relevance in relation to representative use(s)**
None.	

3.1.7 Overview table of the concerns identified for each representative use considered

(If a particular condition proposed to be taken into account to manage an identified risk, as listed in 3.3.1, has been evaluated as being effective, then 'risk identified' is not indicated in this table.)

Representative use		Use "Outdoor" (X[1])	Use "Indoor" (X[1])
Operator risk	Risk identified		
	Assessment not finalised		
Worker risk	Risk identified		
	Assessment not finalised		
Bystander risk	Risk identified		
	Assessment not finalised		
Consumer risk	Risk identified		
	Assessment not finalised		
Risk to wild non target terrestrial vertebrates	Risk identified		
	Assessment not finalised		
Risk to wild non target terrestrial organisms other than vertebrates	Risk identified		
	Assessment not finalised	x	x
Risk to aquatic organisms	Risk identified		
	Assessment not finalised		
Groundwater exposure active substance	Legal parametric value breached		
	Assessment not finalised		
Groundwater exposure metabolites	Legal parametric value breached		
	Parametric value of 10 µg/L(a) breached		
	Assessment not finalised		
Comments/Remarks			

The superscript numbers in this table relate to the numbered points indicated within chapter 3.1.5 and 3.1.6. Where there is no superscript number, see level 2 for more explanation.

(a): Value for non relevant metabolites prescribed in SANCO/221/2000-rev 10-final, European Commission, 2003

3.1.8 Area(s) where expert consultation is considered necessary

It is recommended to organise a consultation of experts on the following parts of the assessment report:

Area(s) where expert consultation is considered necessary	**Justification**
None.	

3.1.9 Critical issues on which the Co RMS did not agree with the assessment by the RMS

Points on which the co-rapporteur Member State did not agree with the assessment by the rapporteur Member State. Only the points relevant for the decision making process should be listed.

Issue on which Co-RMS disagrees with RMS	**Opinion of Co-RMS**	**Opinion of RMS**
Whether commercial Bt strain such as Bta ABTS 1857 can be compared to a pathogenic *B. cereus* strain and that a prediction of a safety level for commercial Bt strains based on information of pathogenic *B. cereus* isolates is reasonable. RMS would like to ask the other member states to look carefully to this issue.	Fresh fruits and vegetables are normally not associated with B. cereus-related diarrhea." This statement should be further supported with literature. For example, in DE a case of food borne disease was reported in 2013 associated specifically to B. thuringiensis. (http://managementbericht.cvuas.de/docs/mb2014.pdf , German only, p.22). Also, since B. cereus cases are not mandatory for reporting, the absence of cases seems a weak basis for such an assumption. Based on EFSA´s BIOHAZ Panel opinion on the presence of Bacillus cereus and other Bacillus spp. in foods (EFSA Journal 2016;14(7):4524) food-borne diseases were associated with concentrations above 105 CFU/g, which was also considered relevant for B. thuringiensis. Since no new data was presented in the RAR allowing the estimation of an alternative safe limit, the reasoning presented is considered as not sufficient to distinguish between both species in terms of their potential to induce foodborne diseases (and also not in terms of routine analytics!). As commented below, the estimated levels of B. thuringiensis following GAP-compliant treatment are likely to exceed 10^5 CFU/g, making this point highly relevant.	RMS is of the opinion that commercial Bt strain such as Bta ABTS 1857 cannot be compared to a pathogenic *B. cereus* strain. The traits responsible for a potential health risk to consumers are highly strain specific. Available strain-specific studies of Bta ABTS 1857 in test animals confirm the absence of toxicity and pathogenicity. Therefore, a prediction of a safety level for strain ABTS 1857 based on information of pathogenic *B. cereus* isolates is not reasonable. The EFSA BIOHAZ panel indicates that most cases of food-borne outbreaks caused by the *B. cereus* group have been associated with concentrations above 10^5 CFU/g and that the levels of *B. cereus* that can be considered as a risk for consumers might be also valid for *B. thuringiensis*. However, as already stated above, this approach is not justified as pathogenic *B. cereus* strains differ significantly from commercial Bt strains in the physiological requirements, environmental behaviour and their toxigenic and pathogenic potential. Based on available information it can be concluded that the risk for consumers due to possible exposure of Bta ABTS 1857 is acceptable. This is confirmed by a lack of case reports in which commercially-used *B. thuringiensis* is directly associated with

		food poisoning (Bta has been used in agriculture for approximately one century; Bta has been approved e.g. in The Netherlands for more than twenty years).

3.2 Proposed decision

[redacted]

3.3 Rational for the conditions and restrictions to be associated with the approval or authorisation(s), as appropriate

3.3.1 Particular conditions proposed to be taken into account to manage the risk identified

Proposed condition/risk mitigation measure	Relevance in relation to representative use(s)
[redacted]	[redacted]

3.4 Appendices

3.4.1 Guidance documents used in this assessment

Guidances applicable at the time of submission of the additional dossier were used in this assessment.

3.5 Reference list

List [in the conventional format] any references specifically cited in Volume 1 (i.e references to underpinning documents such as PPR-Panel Opinions, EFSA conclusions, national documents etc.).