Renewal
Assessment Report

Bacillus thuringiensis ssp. aizawai strain ABTS-1857

Volume 3 – B.7 Residues in or on treated products, food and feed

Rapporteur Member State: The Netherlands

Co-Rapporteur Member State: Germany

Version history

When	What
September 2018	Initial RAR

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B Summary of the data and information

B.7 Residues in or on treated products, food and feed

Bacillus thuringiensis subsp. *aizawai* (Strains ABTS 1857, GC-91) was included in Annex I to Directive 91/414/EEC (2008/113/EC) on 1 May 2009 pursuant to Article 24b of the Regulation (EC) No 2229/2004 and has subsequently been deemed to be approved under Regulation (EC) No 1107/2009 in accordance with Commission Implementing Regulation (EU) No 540/2011 as amended by Commission Implementing Regulation (EU) No 541/2011.

European Food Safety Authority (EFSA) revised the draft review report submitted by the European Commission and EFSA's conclusion was published in the peer review (EFSA Journal 2013; 11(1): 3063).

Italy was designated rapporteur Member State and the DAR was issued in 2007.

The plant protection product XenTari WG is considered representative of uses of the active substance *Bacillus thuringiensis* subsp. *aizawai* Strain ABTS 1857 for the purposes of renewal of the approval of the active substance under EC Regulation 1107/2009 according to Regulation EU 283/2013.

The active substance name has been abbreviated throughout the document to BTa ABTS-1857.

Bacillus thuringiensis subsp. *aizawai* is a gram-positive, spore forming, rod-shaped bacterium that forms a characteristic crystalline protein inclusion. *Bacillus thuringiensis* subsp. *aizawai* is toxic to larvae of some Lepidopteran insects upon ingestion.

The representative product for BTa ABTS-1857 is XenTari® WG, a WG formulation containing 540 g /kg of BTa ABTS-1857 (or approximately 1.17 x 10^{13} viable colony forming units (CFU)/kg product). XenTari® WG is used as a spray for the control of Lepidoptera larvae in outdoor and protected peppers.

B.7.1 Persistence and likelihood of multiplication in or on crops, feedingstuffs or foodstuffs

Bacillus thuringiensis is naturally occurring in the environment and has been isolated from a range of habitats including soil, phylloplane, dust, plant material and insects throughout the world. BTa ABTS-1857 originates from a natural, indigenous wild type, isolated from soil taken from a lawn in Ephraim, Wisconsin (USA) in 1987. BTa ABTS-1857 shows a low acute toxicity via the oral and dermal routes.

Copy from DAR:
B. thuringiensis has been found to have short residual persistence on foliage, with insecticidal activity declining rapidly so that much is lost within one day, and most has gone after a period of a few days (ref. EU DAR, Italy, 2007).

Solar radiation was found to be a key factor in reducing the persistence of populations and the activity of *B. thuringiensis* preparations on the leaf surface. The half-life of *B. thuringiensis* spores on soybean foliage is less than 1 day, and only 8.6% of the initial population was still viable after 1 day. Spore counts returned to background levels after 28 days on soybean and after 14 days on cabbage. Multiplication of *B. thuringiensis* does not seem to occur significantly in natural environments. 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 (Vidal et al. 2013; Tanaka et al 2004; Raymond et al. 2008; Akiba, 1986; West et al. 1985).

Persistence of the spores of B. thuringiensis subsp. kurstaki from Foray bioinsecticide in gleysol and on leaves was studied by Konecka et al. (2014). Two days, 1, 6 and 12 months after application, the number of B. thuringiensis spores on a 4 cm2 surface of leaves were reduced.

In the EFSA BIOHAZ document (EFSA, 2016[1]) an extensive literature search was conducted by EFSA in order to obtain information on the presence and levels of *B. thuringiensis* in food. In general, the information concerning the detection of *B. thuringiensis* in foods was quite scarce, with only a few studies specifically covering this information. In most of the studies, *B. cereus* group organisms were isolated from fruits and vegetables, and differentiation between *B. cereus* and *B. thuringiensis* strains was performed using conventional culture methods and molecular techniques. Enumeration of *B. thuringiensis* levels in food samples was carried out in only a very limited number of studies; however the reported data of the *B. thuringiensis* levels should be interpreted with caution due to the limitations in the analytical methods used.

In one paper (Rosenquist *et al*. (2005)), 70% (28/40) of the *B. cereus* group strains isolated from food matrices contained visible crystals and were therefore identified as *B. thuringiensis*, however it was noted that further studies are needed to clarify the genetic relationship of the isolated strains to commercial *B. thuringiensis*. The levels of *B. thuringiensis* reported in food are very variable, in most cases below 10^3 CFU/g. It was concluded that taking the enterotoxigenic potential into account, together with the fact that *B. thuringiensis* cannot be distinguished from *B. cereus* at the chromosomal level; the levels of *B. cereus* that can be considered as a risk for consumers are also likely to be valid for *B. thuringiensis*. The Rosenquist paper is indicative of the ubiquitous nature and natural occurrence of *B. cereus*. No evidence was provided, nor were there any implications in this study, that any of the strains, *Bacillus thuringiensis* or *B. cereus*-like strains, were involved in any cases or outbreaks of food poisoning. Detailed analysis of the *B. cereus*-like isolates indicated that only 10 (35.7 %) of these strains produced crystals and were positive for *cry1* genes, a characteristic that any isolate originating from a *B. thuringiensis* spray treatment would possess. Of these 10 isolates, 4 were from raw sausage, pasta, bread, and honey. These foods are not from crops that are normally treated with *B. thuringiensis* insecticides. Therefore, only 6 (15%) of the original 40 isolates selected for more detailed taxonomic analysis could have possibly had their origin from *B. thuringiensis* insecticide sprays. Importantly, these six isolates were from red pepper (2), cauliflower (1), leeks (1), salad (1), and figs (1, not usually treated), none of which are typically associated with food poisoning caused by *B. cereus* group species. It should be remembered that *B. thuringiensis* is closely related to *B. cereus* which is found ubiquitously in nature.

Although there is some limited evidence 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.

Fresh fruits and vegetables are normally not associated with B. cereus-related diarrhea. Frederiksen et al. (2006), also cited in the opinion, investigated the occurrence of Bacillus cereus like strains on fresh fruits and vegetables. 128 isolated strains were characterized, of these 50 were classified as B. thuringiensis on the basis of cry genes. RAPD analysis and plasmid DNA profiling revealed that 23 strains were indistinguishable from the active organisms in commercially used products. It has to be underlined that these strains were indistinguishable using the available methods, which are not sufficiently reliable to unequivocally identify a strain. Moreover, this approach is heavily biased as the commercial strains are used as reference. Most probably, they are indistinguishable from commercial as well as various other Bc and Bt strains not considered. 14 isolates were indistinguishable from the B. thuringiensis subsp. kurstaki strain HD1and 9 isolates indistinguishable from the B. thuringiensis subsp. aizawai amongst which strain GC-91. The highest level measured was 10^4 CFU/g in cucumber and tomato. In the samples, also non-commercialised Bt strains were detected as well as other B. cereus like organisms.

However they can be used as ingredients in processed food dishes, may result in conditions that are good for growth. Studies have shown that B. thuringiensis do not multiply in cooked-chilled vegetables or in stored pasteurised vegetable purees and the microbial load of other microorganisms was

[1] EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2016. Scientific opinion on the risks for public health related to the presence of *Bacillus cereus* and other *Bacillus spp*. including *Bacillus thuringiensis* in foodstuffs. EFSA Journal 2016;14(7):4524, 93 pp. doi:10.2903/j.efsa.2016.4524

much higher in these samples (Choma et al. 2000), The microbial load of the cooked chilled vegetable samples was much higher for other microorganisms. The authors indicate that "cooked vegetables packaged after heating spoiled rapidly, presumably because recontamination after cooking occurred in spite of the high care conditions used for packaging, and shelf-life before spoilage was too short to allow a significant growth of B. cereus."

The high nutritional growth requirements for B. thuringiensis were also confirmed by Colla (2010) who tested B. thuringiensis growth in model food systems based on vegetables. The results confirmed that room temperature contributes to the growth dynamics of B. thurin-giensis; the lower temperature of 8°C delayed the onset of the bacterial growth but did not ar-rest the B. thuringiensis biological cy-cle, as can be observed by levelling of the kinetics curve. The CPM model best supported the growth of B. thuringiensis cells; lower pH was the most limiting parameter for microorganism development, as shown by the curve of cell death in pepper cream. The ratio of B. cereus to B. thuringiensis found in this study is similar to the ratio of B. cereus and B. thuringiensis isolated from pasteurised milk (Zhou et al, 2008). Zhou analysed 54 pasteurised full fat milk samples, 40 ice-cream samples, and two green-tea beverage sam-ples. In the full fat milk samples, were 48.1% were found to contain B. cereus, with the average count among the positive samples 11.7 CFU/ml; while 11.1% were found to contain B. thuringiensis with an average count among the positive samples of 4.5 CFU/ml. For ice-cream, the results for B. cereus were 60% and 8.27 CFU/g, and for B. thuringiensis were 2.5% and 3.6 CFU/g respectively. In the two tea beverage samples, the occurrences of B. cereus and B. thuringiensis were 50% and 100%, respectively and the bacterial counts were about 8–10 CFU/ 100 ml.

There is therefore strong evidence to show that B. thuringiensis does not grow and multiply in or on food either when treated with commercial pesticide products or during subsequent processing.

Summary and abstracts of cited literature:

Report:	**KMA 6.1/01**; Konecka E, Baranek J, Bielińska I, Tadeja A, Kaznowski A (2014).
Title:	Persistence of the spores of *B. thuringiensis* subsp. *kurstaki* from Foray bioinsecticide in gleysol and on leaves. J of Science of the Total Environment 472 (2014), 296-301.
Document No:	-
Guidelines:	None
GLP	No

Summary

The aim of this study was to determine how long the spores of *B. thuringiensis* subsp. *kurstaki* HD-1 from a bio-insecticide product persist in soil and on leaf surface after application of the biopesticide in an oak forest. The product was sprayed over a 195-hectare oak forest on the Krotoszyn Plateau in Poland. *B. thuringiensis* was isolated from soil samples and tree leaves taken from randomly chosen sites. *B. thuringiensis* subsp. *kurstaki* HD-1 in the samples was identified upon clonal analysis of the cultured isolates by using the RAPD method.

Site	**Average number of *B. thuringiensis* CFU on 4cm^2 leaf surface**				
	2d before application	**2d after application**	**1m after application**	**6m after application**	**12m after application**
91	0	48.3	3	1	0
92	0	58	5	0.5	0
93	0	38	8	3	0
109	0	43	3	1	0

110	0	76.7	19	1	0
129	0	71	5.5	2	0

B. thuringiensis was not detected on the leaf surface before the biopesticide was applied. Two days after application, the number of *B. thuringiensis* spores on a 4 cm^2 surface of leaves ranged from 43 to 76.7 CFU. Five *B. thuringiensis* strains from each leaf were included in bacterial RAPD typing and all isolates were genetically identical with the B. *thuringiensis* HD-1 strain from the biopesticide use. The average numbers of B. thuringiensis HD-1 on leaves were 56 CFU/4 cm^2. One month after spraying, there was a decrease on the leaf surface with the number of *B. thuringiensis* ranging from 3 to 19 CFU/4 cm^2. Six months after spraying, the number of *B. thuringiensis* ranged from 0.5 to 3 CFU/4 cm^2 on the leaf surface. The RAPD typing showed that B. thuringiensis isolates cultured from all the leaf samples were identical with the B. thuringiensis HD-1 strain from the biopesticide product. Twelve months spraying, the number of *B. thuringiensis* was reduced further and none were isolated from the leaves.

Report:	**KMA 6.1/02**; Frederiksen, K, Rosenquist, H, Jørgensen, K, Wilcks, A. (2006).
Title:	Occurrence of Natural Bacillus thuringiensis Contaminants and Residues of Bacillus thuringiensis-Based Insecticides on Fresh Fruits and Vegetables. J of Applied and Environmental Microbiology (May 2006), Vol. 72, No.5, pp 3435-3440.
Document No:	-
Guidelines:	None
GLP	No

Summary

The main purpose of this study was to determine the occurrence of *B. thuringiensis* on fresh fruits and vegetables for sale in Danish retail shops, including natural contaminants, as well as from residues of *B. thuringiensis*-based insecticides. A total of 128 *B. cereus*-like strains were randomly selected from the positive samples of 991 fresh fruit and vegetable products collected and enumerated for content of *B. cereus*-like bacteria in a previous study (Rosenquist, 2005). The 128 isolates originated from lettuce (*n*= 32), tomatoes (*n* = 8), cucumbers (*n*= 4), peppers (*n* =7), berries (*n*= 17), grapes (*n* =6), herbs (*n* =15), apples (*n*=10), root vegetables (*n* = 19), and other products (*n* =10). Of these strains, 39% (50/128) were classified as *Bacillus thuringiensis* on the basis of their content of *cry* genes determined by PCR or crystal proteins visualized by microscopy. Random amplified polymorphic DNA analysis and plasmid profiling indicated that 23 of the 50 *B. thuringiensis* strains were of the same subtype as *B. thuringiensis* strains used in commercial bio-insecticides. The commercial strains were primarily isolated from samples of tomatoes, cucumbers, and peppers. Fresh fruits and vegetables are normally not associated with *B. cereus*-related diarrhoea. However, used as ingredients, these products may contaminate complex food dishes, e.g. starchy dishes, in which there are good conditions for growth, especially if the final dishes are improperly cooled after heat treatment.

Report:	**KMA 6.1/03**; Choma, C, Guinebretiére, MH, Carlin, F, Schmitt, P, Velge, P, Garnum, PE. & Nguyen-The, C. (2000).
Title:	Prevalence, characterisation and growth of Bacillus cereus in commercial cooked chilled foods containing vegetables. J of Applied Microbiology 2000, 88: 617-625.
Document No:	-
Guidelines:	None
GLP	No

Summary

The populations of *Bacillus cereus* in two types of cooked chilled foods containing vegetables were studied: vegetable purees produced for the retail market (heated in their final package at 80°C for 10±20 min) and cooked vegetables produced for caterers (cooked at 98°C for 10±15 min and then packaged). *B. cereus* was detected in 20% of vegetable purees pasteurised in their original package at levels less than 10 CFU/g. The products were subsequently stored at room temperature. When spoilage was detected in products stored at room temperature (20±25°C, between four and 12 days), 70% of samples were found to be positive. After 20 days storage at 10°C, 50% of samples were positive for *B. cereus* at levels of containing 10^4 to 10^6 CFU g/1, but showed no signs of spoilage.

Isolates of *B. cereus* were further characterised as shown in the table below:

API Profiles	**Pasteurised pureés**				**Cooked chilled vege-tables†**	**Total**
	Un-stored product	**Stored at 4°C**	**Stored at 10°C**	**Stored at 20-25°C**		
B. cereus 1	1	2	2	6	21	32
B. cereus 2ǂ	0	0	0	0	8	8
B. cereus 1/ *B. mycoides*	1	0	13	13	1	37
B. cereus / B. *thuringiensis*	1	0	0	0	0	1
B. cereus / *B. laterosporus*	0	0	3	3	0	3
B. mycoides	0	0	0	0	0	1
B. cereus / *B. anthracis* §	0	0	0	0	1	1

All strains had been previously confirmed as *Bacillus cereus* by ISO 7932 and NF V 08-058 procedures
† Numbers of *B. cereus* in cooked chilled vegetables were always than 100 CFU g^{-1}
ǂ Isolates did not hydrolyse starch
§ Isolates were haemolytic

The *thuringiensis* strain was not present in the cooked chilled vegetables or in any of the stored vegetable purees. There was no growth of *B. thuringiensis* in the stored vegetable purees. The microbial load of the cooked chilled vegetable samples was much higher for other microorganisms. The authors indicate that "cooked vegetables packaged after heating spoiled rapidly, presumably because recontamination after cooking occurred in spite of the high care conditions used for packaging, and shelf-life before spoilage was too short to allow a significant growth of *B. cereus*."

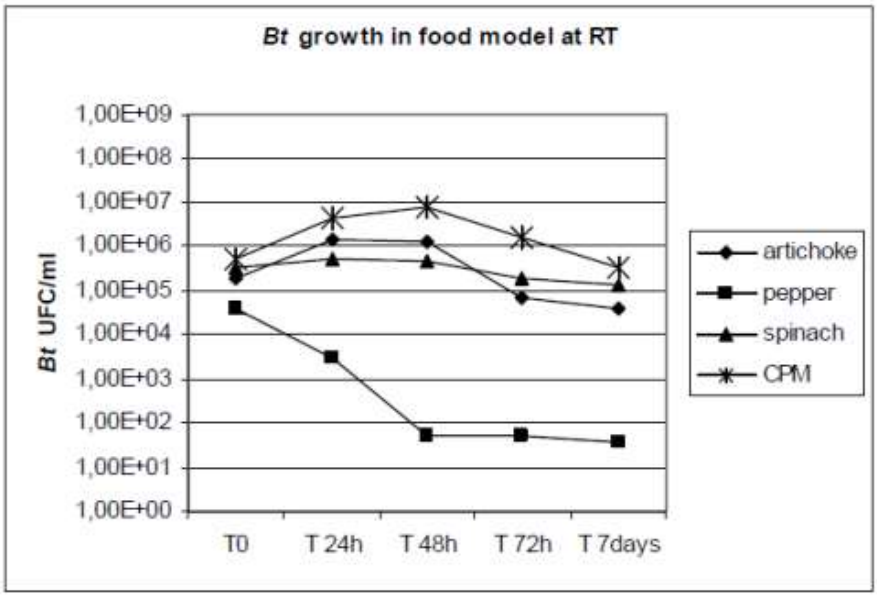
Report:	**KMA 6.1/04**; Colla, Francesca (2010).
Title:	Study of Bacillus thuringiensis behaviour in food environment by genome-wide transcriptome analysis. Chapter 3. University of Verona.
Document No:	-
Guidelines:	None
GLP	No

Summary

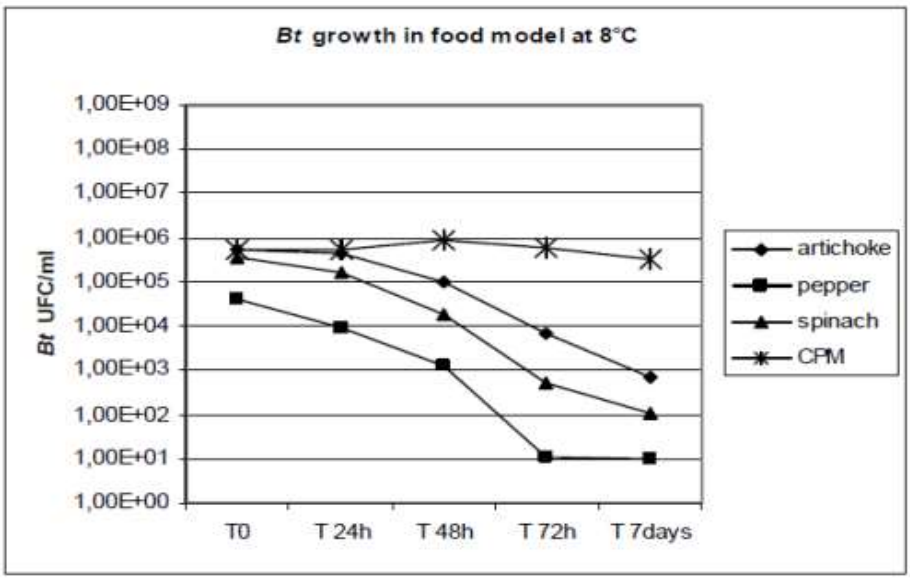
A vegetable based food model was developed to assess the behaviour of *B. thuringiensis* spores in food, after the simulation of industrial processing treatments. Four types of different vegetables matrix were tested for the ability to support the development of commercial *B. thuringiensis* spores and growth of vegetative cells. Three commercial UHT vegetable creams based on pepper, artichoke and spinach, and one pasteurized purée, compounded from courgettes, potatoes and milk (referred to as the CPM model) were tested using *Bacillus thuringiensis kurstaki* isolated from commercial bio-pesticide products. The test matrices were inoculated with *B. thuringiensis* to a concentration of 10^7 CFU/ml and stored for 1 week at 8°C and room temperature, after which growth was monitored by plate counting.

B. thuringiensis growth curves at room temperature and 8°C are presented below.

A



B



The results confirmed that room temperature contributes to the growth dynamics of *B. thuringiensis;* the lower temperature of 8°C delayed the onset of the bacterial growth but did not arrest the *B. thuringiensis* biological cycle, as can be observed by levelling of the kinetics curve. The CPM model best supported the growth of *B. thuringiensis* cells; lower pH was the most limiting parameter for microorganism development, as shown by the curve of cell death in pepper cream.

Report:	**KMA 6.1/06**; Zhou, G, Yan, J, Dasheng, Z, Zhou, X, Yuan, Z. (2008).
Title:	The residual occurrences of *Bacillus thuringiensis* bio pesticides in food and beverages. Int. J of Food Microbiology 127 (2008) 68-72.
Document No:	-
Guidelines:	None
GLP	No

Summary

54 pasteurised full fat milk samples, 40 ice-cream samples, and two green-tea beverage samples were analysed. In the full fat milk samples, were 48.1% were found to contain *B. cereus*, with the average count among the positive samples 11.7 CFU/ml; while 11.1% were found to contain *B. thuringiensis* with an average count among the positive samples of 4.5 CFU/ml. For ice-cream, the results for *B. cereus* were 60% and 8.27 CFU/g, and for *B. thuringiensis* were 2.5% and 3.6 CFU/g respectively. In the two tea beverage samples, the occurrences of *B. cereus* and *B. thuringiensis* were 50% and 100%, respectively and the bacterial counts were about 8–10 CFU/ 100 ml.

Sources of *B. thuringiensis* isolates and concentration of *B. cereus* group organisms in each product

Isolates	**Sources**	**Concentrations of B. cereus group strains in samples (CFU ml^{-1} or CFU g^{-1})**		
		Commercial *B. thuringiensis*	**Non – commercial**	***B. cereus***

			B. thuringiensis	
Bt1	Spring milk - 22	3.0	ND	43
Bt2	Spring milk - 24	3.6	ND	7.4
Bt3-6	Spring milk - 25	ND	11	36
Bt7	Spring milk - 46	ND	3.6	11
Bt8	Spring milk - 47	ND	3.0	3.0
Bt9	Ice cream -2	ND	3.6	3.0
Bt10	Autumn milk - 30	ND	3.0	3.6
TYA1-7	Tea beverage A	<1	<1	ND
TYB3-5	Tea beverage B	ND	<1	<1

ND = not detected

B.7.2 Further Information required - Exposure to consumers

In B.6 Bacillus thuringiensis and food poisoning is discussed in detail. *B. cereus* and *B. thuringiensis* strains are very similar and, consequently have not been distinguished in cases of food poisoning using routine methodology. The EFSA Panel on Biological Hazards (BIOHAZ) has recently concluded that the levels of *B. cereus* considered to be a consumer risk for are $>10^5$ organisms/g food (although this will be strain-specific). EFSA further recommend the use of genome sequencing to discriminate *B. cereus* from *B. thuringiensis* in food poisoning cases (EFSA, 2016). The document notes that there is no definitive evidence for the role of these toxins (alone or in combination) in the diarrhoeal syndrome. Although based on the discussion described in B.6 RMS concluded that the presence of Bacillus thuringiensis ssp. aizawai (strain ABTS 1857) on food at levels similar to those reported to cause food poisoning by pathogenic strains of B. cereus does not have any health implications. Recent data support the long-held view that Bt and especially the strains used in Bt biopesticides are very safe for humans.

B.7.2.1 Non-viable residues

The crystal proteins are not toxic or pathogenic to humans, plants, and most animals except for larvae of target and non-target species belonging to the insect order Lepidoptera. Therefore toxicological end points are not required.

There is, no evidence that B. thuringiensis has the genetic determinants for the production of emetic toxins (cereulide) or β -exotoxin. In B.2 the production of metabolites is discussed in detail.

Some members of the *Bacillus* group can cause diverse diseases while others appear to be non-pathogenic; *B. cereus* is an opportunistic human pathogen involved in food-related infection outbreaks and considered as an important food contaminant. Some strains of *Bacillus thuringiensis* are thought to produce the same enterotoxins known from *Bacillus cereus* to cause diarrhoea in humans; however the majority of *B. thuringiensis* strains investigated have been shown not to produce these enterotoxins. Genetic sequencing of commercial *B .thuringiensis* strains has shown the absence of some of the genes encoding diarrheal toxins (such as CytK1) as well as the lack of operons required to express some of the tripartite genes encoding other enterotoxins. Therefore although there are genetic similarities between *B. thuringiensis* strains and the *B. cereus* group, there are key differences in the presence and expression of the toxin genes.

It can be concluded that there is no evidence for enterotoxin production under normal growth conditions for BTa ABTS-1857. Even if enterotoxin production does 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 known pathogenic *B. cereus* strains.

The foodborne pathogen *Bacillus cereus* can cause diarrhoeal food poisoning by production of enterotoxins in the small intestine. The prerequisite for diarrhoeal disease is therefore survival during gastrointestinal passage. Vegetative cells have been seen to not survive gastric acid and bile during in vitro gastrointestinal transit. The studies presented on *B. thuringiensis ssp. Aizawai* through a simulated gut present no germination or growth of vegetative cells.

Therefore given that the production of enterotoxin is unlikely to occur, the risk from non-viable residues from BTa ABTS-1857 is not of concern.

It has been demonstrated that BTa ABTS-1857 does not produce significant quantities of β-exotoxin and cytolytic proteins or enterotoxins during the production process (see B.1).

B.7.2.2 Viable residues

The results of acute mammalian toxicity studies show that Bacillus thuringiensis subsp. aizawai, Strain ABTS-1857 does not pose a toxic, infective or pathogenic hazard to humans. The spores are not toxic or pathogenic to humans, plants, and most animals except for larvae of target and non-target species belonging to the insect order Lepidoptera. Therefore toxicological end points are not required.

BTa ABTS-1857 is an insect pathogen and does not have the propensity for growth under environmental conditions that would apply to strains of the B. cereus group. The EFSA BIOHAZ document indicates the occurrence of Bacillus species in raw materials used for food processing or in prepared foods such as soups, sauces, puddings, milk, meat and vegetables, is generally below 10^5 cells/g or mL food. It was concluded in the EFSA BIOHAZ document that B. cereus group vegetative cells or spores must have the opportunity to multiply in the food chain environment to enable them to cause food spoilage or poisoning. As demonstrated in this section there is strong evidence to show that B. thuringiensis does not grow and multiply in or on food.

Pedersen et al (1995) investigated the field population dynamics of a Bacillus thuringiensis ssp. kurstaki isolated from cabbage leaves and found that germination of B. thuringiensis ssp. kurstaki was not demonstrated in any leaf or soil sample. The half-life time of B. thuringiensis ssp. Kurstaki was estimated to be 16 hours confirmed by the half-life of B. thuringiensis ssp. kurstaki strain ABTS-352 spores on maize leaves (Haddad et al. 2005).

Studies on lettuce, tomatoes and peppers were conducted to determine the concentration of spores of BTa ABTS 1857 following application of XenTari® WG. Tomatoes and peppers both belong to the fruiting vegetable, solanacea crop group so therefore the data on tomatoes is relevant to uses on peppers. Residues trials demonstrate spore counts of BTa ABTS-1857 do not expected to exceed the level 1 x 10^5 CFU/g fresh weight after field application when crops are treated according to the proposed GAP as Stephan (2013a) found that when XenTari® was applied on tomatoes in the laboratory (in glasshouse protected uses), an average concentration of 5 x10^5 CFU/g was determined. The values ranged from 5.4 x 10^4 to 2.1 x 10^6 CFU/g. Moreover Stephan (2013b) showed degradation of spores over time under protected glasshouse conditions with a reduction of between 46 – 77% of the initial spore concentration after a week after single or multiple applications of XenTari® WG on tomatoes.

Bacillus thuringiensis is naturally occurring in the environment and has been found to have short residues persistence on foliage, with insecticidal activity declining rapidly. Under field conditions the spores will be inactivated in a short time because they are highly sensitive to UV light. Solar or ultra violet radiation, temperature, humidity, wind and rain can limit persistence of B. thuringiensis spores (Brar et al. 2006). Spores can be rapidly inactivated by UV radiation and sunlight. Survival can drop more than 90% within 20 minutes of exposure to sunlight (Brar et al. 2006; Griego and Spence 1978). In glasshouses it is thought that glass acts as a barrier to UV light therefore it is possible that spores will not be inactive as quickly as in the field. For this reason glasshouse (protected) uses are considered to be the "worst case" for the potential of significant residues of BTa ABTS 1857 after the use of XenTari® WG.

None of the treated tomatoes (5 applications) were found to contain a spore count greater than 10^5 CFU/g fresh weight; the total count at after 5 days was approximately 10^3 CFU/g fresh weight in a study performed by Ehrhardt (2016). This is confirmed by Wagner (2016) in which the overall mean and median counts for tomatoes and peppers were well below the standard of 1 x 10^5 CFU/g fresh weight. These were data from trials conducted in 2015 indicating that residues levels in peppers and tomatoes are comparable. Applications were made at rates equivalent to or greater than the maximum individual dose rate per treatment.

The decline trial data where five applications were taken included a sampling point before the last applications. From the results it can be seen that there is decline in residues over time. This is supported by the 2016 decline study. This means that residues decline between applications and it is the last application only that will have a significant impact on the final residue level. Therefore trials conducted using three or five applications can be considered relevant to support a use where more applications are made.

Smaller sized crop varieties were included in the experiments which would be expected to lead to higher concentrations of "residues" of spores; the results indicate that this is the case. Nevertheless none of the samples at any time point were found to contain spore concentrations greater than 1 x 10^5 CFU/g fresh weight even when applications were made at twice the maximum individual rate per treatment, 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.

Summary and abstracts of cited literature:

Report:	**KMA 6.2.2/01** Pedersen, Damgard and Eilenberg (1995).
Title:	Dispersal of Bacillus thuringiensis var. kurstaki in an experimental cabbage field, Can. J. of Microbiol. 41 (2) 118-125) **Assessed in EU DAR for Bt kurstaki Strain ABTS-351, (Denmark, 2008)**
Document No:	-
Guidelines:	None
GLP	No

Summary

The field population dynamics of a *Bacillus thuringiensis ssp. kurstaki* isolated from cabbage leaves was investigated. Two experimental cabbage plots were spray inoculated with a suspension of sporulated bacteria containing 6.5×10^7 colony-forming units (CFU)/mL or with a sterile medium control. To study transport between phyllosphere and soil, plastic covering was used during spraying to ensure that the *B. thuringiensis ssp. kurstaki* was applied only on leaves (8.5×10^6 CFU/g) or on soil (1.2×10^4 CFU/g). Germination of *B. thuringiensis ssp. kurstaki* was not demonstrated in any leaf or soil sample.

The study was assessed in the EU DAR for Bt kurstaki strain ABTS-351, (Denmark, 2008) as follows;

Dispersal of *Bacillus thuringiensis* var. *kurstaki* in an experimental cabbage field, (Pederesen, J.C, Damgaard, P.H., Eilenberg, J & Hansen, B.M., 1995)

In this study dispersal of *B. thuringiensis* subsp. *kurstaki* in an experimental cabbage field were examined. In the experiment the strain *B. thuringiensis* strain DMU67R was used, which is a spontaneous mutant of the organism *B. thuringiensis* subsp. *kurstaki* strain DBt14.

Experimental plots were planted with cabbage. Larvae of *P.brassicae*, to which B. *thuringiensis* subsp. *kurstaki* is pathogenic, were introduced to each cabbage plants 1-3 days before application. To study dispersal between soil and the phyllosphere, polyethylene covering was carefully applied immediately before spraying to protect either plants or soil from treatment. Spraying was carried out when the plants had 7-10 regular leaves. In the control plot DMU67R was never detected in samples of soil or leaves.

A leaf sample consisted of a portion of each of five leaves among the lower nine leave from each of five cabbage plants. Samples of leaves were taken regularly throughout about 1 year as duplicate samples. Soil samples were taken from the top 2-cm layer regularly throughout about 1 year. All samples were placed on ice and processed within 6 hours. Firstly the samples were homogenised followed by enumeration. Both leaf and soil samples were diluted in Winogradskys salt solution and an aliquot of this solution was heat-treated followed by plating in duplicate on Luria-Bertani agar with rifampicin. The plates were incubated overnight at 30° C. The identities of the selected colonies were verified using two different PCR methods. No other bacteria than DMU67R were identified on the selective plates.

For soil application the strain DMU67R was introduced at 1.2 x 10^4 CFU/g soil. After minor fluctuations during the first 28 days the number gradually decreased to 2.3 x 10^3 CFU/g soil after a year. Using linear regression analysis on the data from day 28 onwards a half-life time of 120 days was established. After soil application 3.9 x 10^2 CFU/g was observed on the leaves and this numbers remained until day 28. A few colonies of DMU67R were observed at day 49 and none at day 135. However on day 336 about 87 CFU/g were detected on young leaves samples 5-10 cm above ground.

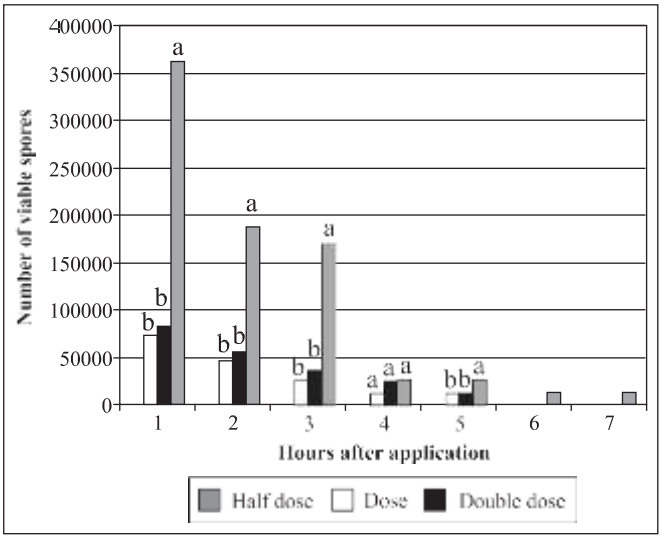
When the strain DMU67R was applied to the leaves 8.5 x 10^5 CFU/g leaf was detected 1 hour after spraying. The amount of DMU67R decreased by five orders of magnitude during the following 28 days and was not detected after day 28. During the first 28 days the cabbage leaves increased their dry mass, which could explain about 0.7 log units of the decrease. Linear regression analysis showed that the initial half-life of DMU67R for day 0-7 was 16 hours.

As can be seen from this study *B. thuringiensis* subsp. *kurstaki* can be dispersed in a minor extent from the soil to the lower leaves of the cabbage plants. The study also shows that no multiplication takes place in neither the soil nor the leaves. The half-life time of *B.thuringiensis* on cabbage was estimated to be 16 hours.

Report:	**KMA 6.2.2/02** Haddad, M, Polanczyk, R, Alves, S & de Olivera Garica, M. (2005).
Title:	Field Persistence of Bacillus thuringiensis on maize leaves. Brazilian J of Microbiology, 36: 309-314
Document No:	-
Guidelines:	None
GLP	No

Summary

The half-life of *B. thuringiensis ssp. kurstaki* strain ABTS-352 spores on maize leaves was investigated by using three spray concentrations (half, normal and double doses) of a commercial formulation. In each plot three leaves in the upper part of three plants were randomly selected and samples collected 3 to 72 hours after treatment. The field persistence was determined using an exponential model, linearized by a logarithmic transformation of viable spore numbers in time. There were no significant differences (P = 0.05) in half-lives between the doses: 18.2 hours for half-dose, 16.5 hours for normal dose and 13.6 hours for double dose.



Average number of *Bacillus thuringiensis* viable spores (mL^{-1}) on maize leaves after commercial spray application.

Report:	**KMA 6.2.2/03**; Stephan D; 2013a
Title:	Investigation of the spore concentration of Bacillus thuringiensis on marketable Salanova Lettuce Heads after application with XenTari® WG
Document No:	-
Guidelines:	None
GLP	No

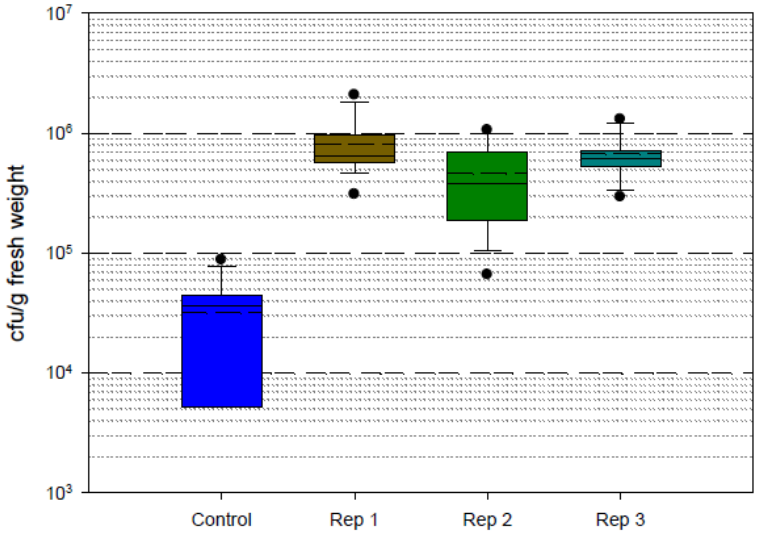
In a laboratory experiment, lettuce plants (variety Salanova) provided from a commercial grower were potted up individually and allowed to acclimatise in a glasshouse. The lettuce plants were individually treated with XenTari® WG at an application rate equivalent to 1000 g XenTari® WG per ha in 400 L water. The experiment was repeated three times with three replicates, and three samples per replicate.

Control plants were sprayed with sterile tap water. On the day after application samples were taken for analysis. Approximately 10g of leaf was suspended in isotonic solution and shaken for 10 minutes. The spore suspension was heated for 20 minutes at +80°C and relevant dilutions were plated on TSA (Tryptic Soy Agar) using a spiral plater. After 20 hours incubation at 25°C the number of colony forming units (CFU) per gram fresh weight was calculated.

Spore concentrations in control samples were found at levels up to 9 x 10^4 CFU/g (Figure 1). The lettuce plants were provided from a commercial grower at a mature growth stage therefore it cannot be excluded that this high value was caused by treatment of *Bacillus thuringiensis* products during cultivation. When XenTari® was applied in the laboratory, an average concentration of 5 x10^5 CFU/g was determined. The values ranged from 5.4 x 10^4 to 2.1 x 10^6 CFU/g.

The results show that for lettuce, the threshold of 1 x 10^5 CFU/g can be obtained within 24 hours of a single application.

Figure MMA 6.2.2-1: Box plot of *Bacillus thuringiensis* spore concentration on lettuce before (water control) and after treatment with XenTari® WG (Rep 1-3: Three independent replicates).



Report:	**KMA6.2.2/04**; Stephan D; 2013b
Title:	Summary of investigations on residues of *Bacillus thuringiensis* on lettuce and tomato
Document No:	-
Guidelines:	None
GLP	No

A series of three experiments were conducted to investigate the viable residues of Bacillus *thuringiensis* on tomato.

In the first experiment, conducted in a laboratory, ripe tomatoes were treated at an application rate equivalent to 1000 g XenTari® WG per ha in 600 L water (the maximum dose rate per treatment according to the proposed GAP). Immediately after application, the tomatoes were frozen at -20°C.

The second experiment was conducted in four individual experimental glasshouses in Germany. In each glasshouse five applications of XenTari® WG were made at a rate of 2 kg XenTari® WG/ha

applied to run off with an interval of seven days between applications (twice the maximum dose rate per treatment according to the proposed GAP). The water volume used was 1200 l/ha. Samples of approximately 1 kg of fruits were collected before the first application, before and after the last application and 1, 2, 3, and 7 days after the last application in each plot. Additionally, samples were taken from the untreated control. Immediately after sampling, the tomatoes were frozen at -20 °C.

In the final experiment was conducted under commercial growing conditions in a glasshouse in Germany. One application was made at a rate of 2 kg XenTari®/ha applied to runoff. The water volume used was 860 l/ha (twice the maximum dose rate per treatment according to the proposed GAP). For some plants the whole tomato plant was treated, and in others only the upper part of the plant (leaves and young tomato fruits) was treated. Samples of approximately 1 kg of fruits were collected before the first application, before and after the last application and 1, 2, 3 and 7 days after the last application. Immediately after sampling, the tomatoes were frozen at -20°C.

Analysis

Tomatoes were transferred into a bottle with a defined volume of isotonic solution and were shaken for a defined time. Afterwards, samples of the liquid were taken and were heat treated for 20 minutes at +80°C. Relevant dilutions were plated in triplicate onto TSA using a spiral plater. After 20 hours incubation at 25°C the number of colony forming units (CFU) per gram fresh weight was calculated.

Results

In the first experiment from a single application the total spore count was found to be 5×10^4 CFU/g on a fresh weight basis. Results from the second and third experiments are present in Table MMA 6.2.2-1 and Table MMA 6.2.2-2, respectively.

Table MMA 6.2.2-1: Colony forming units (CFU/g) following five applications of XenTari® WG on glasshouse grown tomatoes.

Sampling		**CFU/g fresh weight**			
Date	**PHI (days)**	**Plot 1: Vine tomatoes**		**Plot 2: Cherry tomatoes**	
		Control	**Treated**	**Control**	**Treated**
18.07.2013	Before 1st application	870	-	10.6	-
13.08.2013	0-	-	2.7×10^4	-	3.8×10^4
13.08.2013	0+	49.3	4.9×10^4	44.3	5.9×10^4
14.08.2013	1	87.3	4.5×10^4	0.0	7.0×10^4
15.08.2013	2	0.0	3.2×10^4	24.2	5.8×10^4
16.08.2013	3	4.65	2.4×10^4	0.0	4.6×10^4
20.08.2013	7	48.9	3.3×10^4	0.0	4.5×10^4
Sampling		**CFU/g fresh weight**			
Date	**PHI (days)**	**Plot 1: Vine tomatoes**		**Plot 2: Cherry tomatoes**	
		Control	**Treated**	**Control**	**Treated**
24.07.2013	Before 1st applica-	97.7	-	0.0	-

	tion				
20.08.2013	0-	-	3.3×10^4	-	3.8×10^4
20.08.2013	0+	22.4	5.8×10^4	13.6	8.5×10^4
21.08.2013	1	170	4.6×10^4	0.0	6.6×10^4
22.08.2013	2	6.9	3.9×10^4	28.7	5.6×10^4
23.08.2013	3	11.2	3.6×10^4	91.8	5.4×10^4
27.08.2013	7	19.9	2.8×10^4	14.6	6.9×10^4

Table MMA 6.2.2-2: Colony forming units (CFU/g) following one application of XenTari® WG on commercially grown glasshouse tomatoes.

Sampling		**CFU/g fresh weight**		
Date	**PHI (days)**	**Control**	**Treated: Whole plant**	**Treated: Upper plant**
17.09.2013	0-	33	130	59
17.09.2013	0+	170	2.1×10^4	1.9×10^4
18.09.2013	1	87	1.8×10^4	3.3×10^3
19.09.2013	2	40	1.4×10^4	3.1×10^3
20.09.2013	3	190	10	1.1×10^3
23.09.2013	7	110	1.3×10^4	1.2×10^3

In the experiment where five applications were made, the concentration of spores on tomato fruits ranged between 4.9×10^4 and 8.5×10^4 CFU/ g fresh weight. None of the samples contained spore concentrations greater than 1×10^5 CFU/g fresh weight.

Decline data taken after single or multiple applications of XenTari® WG showed degradation of spores over time under protected glasshouse conditions with a reduction of between 46 – 77% of the initial spore concentration after a week.

For the third experiment a significant reduction of the spore count on treated fruits was seen when only the upper parts of the tomato plant were treated.

Report:	**KMA 6.2.2/05**; Ehrhardt J ; 2016
Title:	Residue study (decline) on tomato following three foliar applications of XenTari® WG under protected conditions in Germany 2015
Document No:	VP15-1-74
Guidelines:	EEC document 7029/V1/95 rev. 5, 1997, Appendix B working document 1607/V1/97, rev. 2, 1999: General recommendation for the design, preparation and realisation of residue trials The Principles of Good Laboratory Practice, Chem G 25.07.1994, §19, Annex 1 (BGBL 21, I, 2001, p. 843-855) OECD-Principles of Good Laboratory Practice, No. 4: Quality Assurance and GLP (as revised in 1999), ENV/JM/MONO (1999)20, Paris 2002 The Application of the GLP Principles to Field Studies, OECD Consensus Document, 6, revised, ENV/JM/MONO (1999) 22, Paris 2002 The Application of the OECD Principles of GLP to the Organisation and Management of Multi-site Studies, OECD Consensus Document, 13, ENV/JM/MONO (2002) 9 Rückstandsversuche, Teil 1 Prüfungen an Pflanzen, A: Allgemeiner Teil, B: Spezieller Teil, IVA-Guideline, Industrieverband Agrar e. V. 1992 Genehmigungsverfahren für Pflanzenschutzmittel: XenTari. G18 024426-00/04-008. Bundesamt für Verbraucherschutz und Lebensmittelsicherheit. 16.07.2014, p. 6-7
GLP	Yes

One trial was conducted in a greenhouse in Germany. Tomato plants were treated with three applications of XenTari® WG at a rate equivalent to 1.5 kg XenTari® WG/ha with an interval of 5 – 7 days between applications. The water volume used was 900 L/ha (based on a plant height of 50 - 125 cm).

Samples of tomato fruits were taken immediately after the final application (day 0), and 1, 3 and 5 days after the final application. The specimens were stored frozen (-18°C) before analysis.

Analysis

Samples were initially analysed according to DIN EN ISO 7932 BVL L00.00-33. This method determines *Bacillus cereus* and does not distinguish between *B. cereus* and *B. thuringiensis*:

Samples were thawed and rinsed with demineralised water, the pooled rinse water from all fruits in one sample were combined. An aliquot (0.1 ml) of the combined rinsing was plated onto Mannitol Egg Yolk Polymyxin Agar (MYP-Agar) and incubated at 30 ±1°C for 48 hours. After incubation, colonies fitting the criteria for *B. thuringiensis* were counted and up to 5 representative colonies transferred onto a fresh MYP-Agar plate. After incubation at 30 ±1°C for 18-24 hours at least one colony was transferred to Columbia Sheep-Blood Agar and incubated for 20-24 hours at 30 +/- 1 to test for haemolytic colonies. The colonies are confirmed as *B. thuringiensis* if the following criteria are met:

Table MMA 6.2.2-3: Criteria for biological assessment of *B. thuringiensis* spores.

Agar	Criteria
Mannitol Egg Yolk Polymyxin Agar	Pink to white colonies with precipitation zone
Columbia Sheep-Blood Agar	Positive. Diameter of haemo-lytic zone may vary.

A second analysis was conducted at a different laboratory:

> Tomatoes were transferred into a bottle with 250 ml of 0.9% sodium chloride and were shaken for 10 minutes. Relevant dilutions were plated in triplicate onto MYP-Agar using a spiral plater. After 20-24 hours incubation at 25°C the number of colony forming units (CFU) per gram fresh weight was calculated.

Results

Results from the first and second analysis experiments are present in Tables MMA6.2.2-4a and b and Table MMA6.2.2-5 respectively.

Table MMA 6.2.2-4a: FIRST ANALYSIS: Colony forming units (CFU/g) following three applications of XenTari® WG on glasshouse grown tomatoes – 48 hour incubation on MYP- Agar.

Sample	CFU per plate		Mean CFU/mL	Mean CFU/g fresh weight
	Replicate 1	Replicate 2		
Untreated DAY 0	0	0	0	0
Untreated DAY 1	6	5	0	0
Untreated DAY 3	1	5	0[1]	0
Untreated DAY 5	0	0	0	0
Treated DAY 0	210	178	1940	181
Treated DAY 1	31	20	255	23
Treated DAY 3	0	0	0	0
Treated DAY 5	0	0	0	0
Control[2]	3	0	15	-

[1] Found colonies were not confirmed as *B. thuringiensis*, therefore mean CFU/ml is considered to be 0
[2] Control samples indicated some ubiquitous *Bacillus Spp.* spores present, therefore the significant level of CFU was set at 4x control values i.e. 4 x 15 = 60 CFU/ml.

Table MMA 6.2.2-4b: Transferred colonies on MYP- Agar and Columbia Sheep Blood Agar (CSB-Agar).

Sample	**Transferred colonies**	**MYP Agar**				**CSB Agar**	**Confirmed *B. thuringiensis* colonies**
		Colour of colonies			**Colonies with clear precipitation zone**	**Colonies with haemolytic zone**	
		White	**Pink**	**Other**			
Untreated DAY 0	-	-	-	-	-	-	-
Untreated DAY 1	10	/	/	/	/	-	X
Untreated DAY 3	6	6	-	-	6	3	X
Untreated DAY 5	-	-	-	-	-	-	-
Treated DAY 0	10	6	4	-	10	10	10
Treated DAY 1	10	10	-	-	10	10	10
Treated DAY 3	-	-	-	-	-	-	-
Treated DAY 5	-	-	-	-	-	-	-

- = Not determined
/ = No growth on medium
X = Not confirmed as *B. thuringiensis*

The first analysis of specimens of the treated plots resulted in values between 0 and 181 CFU/g fresh weight. Since these low values were found in the specimens, even directly after the application, the analysis was repeated. To improve the removal of the *B. thuringiensis* spores from the fruit surfaces, the procedure of rinsing the fruits was modified for the second analysis.

Table MMA 6.2.2-6: SECOND ANALYSIS: Colony forming units (CFU/g) following three applications of XenTari® WG on glasshouse grown tomatoes – 24 hour incubation on MYP- Agar

Sample	Mean CFU/g fresh weight
Untreated DAY 0	59
Untreated DAY 1	0
Untreated DAY 3	11
Untreated DAY 5	26
Treated DAY 0	56934
Treated DAY 1	0
Treated DAY 3	43623
Treated DAY 5	783

The results of the second analysis in treated tomatoes were 56934 CFU/g fresh weight 0 days after the last application, 43623 CFU/g fresh weight three days after the last application and 783 CFU/g fresh weight 5 days after the last application.

The analysis of the specimens from untreated plots showed average means between 0 and 59 CFU/g fresh weight. The samples taken 1 day after application showed no spores in both the treated and untreated samples. The reason for these results was not identified. None of the treated tomatoes were found to contain a spore count greater than 10^5 CFU/g fresh weight; the total count at after 5 days was approximately 10^3 CFU/g fresh weight.

Report:	**KMA6.2.2/06**; Wagner A ; 2016
Title:	Trials of Residue on Tomatoes and Peppers, Part of the Residues Research by the Minor Uses Working group
Document No:	-
Guidelines:	Field trials were conducted according to: EEC document 7029/V1/95 rev. 5, 1997, Appendix B working document 1607/V1/97, rev. 2, 1999: General recommendation for the design, preparation and realisation of residue trials
GLP	Yes (field part)

Trials on tomatoes and peppers were conducted in glasshouses in Germany in 2015. Five trials were conducted on tomatoes and seven trials on peppers. Tomato plants were treated with three applications of XenTari® WG at a rate equivalent to 1.5 kg XenTari® WG/ha with an interval of 5 – 7 days between applications. The water volume used was 1200 L/ha.

Samples of tomato fruits were taken immediately after the final application (day 0) and 1, 3 and 5 or 7 days after the final application. The specimens were stored frozen (≤ -18°C) before analysis.

Pepper plants were treated with three applications of XenTari® WG at a rate equivalent to 1.5 kg XenTari® WG/ha with an interval of 5 – 7 days between applications. The water volume used was 600 L/ha. Samples of peppers were taken immediately after the final application (day 0) and 1, 3 and 5 days after the final application. The specimens were stored frozen (≤ -18°C) before analysis.

All samples were analysed within six months of treatment.

Analysis

Samples were analysed to determine the number of presumptive *bacillus cereus* present. The methodological approach was based on the following testing procedures:

> Amtliche Sammlung von Untersuchungsverfahren (Official Compendium of Test Methods) in accordance with § 64 LFGB (Food-Contact Applications Act)
>
> Food Trials – Horizontal Procedure for Counting Presumptive Bacillus Cereus – Colony Counting Method at 30 °C

Stems were removed from the frozen tomato samples prior to analysis. Pepper samples were thawed to allow the removal of stems, cores, ribs and most of the seeds. All samples were weighed after this preliminary preparation and before analysis.

Weighed samples were transferred into a bottle with 350 ml of 0.9% sodium chloride solution and were shaken for 10 minutes. For cherry tomato samples 250 ml of 0.9% sodium chloride solution was used. Relevant dilutions were plated in triplicate onto MYP-Agar using a spiral plater. After 20-24 hours incubation at 25°C the number of colony forming units (CFU) per gram fresh weight was calculated.

Results

Results are presented in Table MMA6.2.2-7 for tomatoes and Table MMA6.2.2-8 for peppers.

In the tomato trials, none of the mean spore counts exceeded 1×10^5 CFU/g fresh weight; total counts 5-7 days after the final treatment ranged from 1.0 to 4.9 x 10^4 CFU/g fresh weight.

In the pepper trials, the mean spore counts were all below1×10^5 CFU/g fresh weight with the exception of 1 trial (LR-G-15-FG-I-02-HAM-01). In this trial the total count was 1.1 x 10^5 CFU/g after 1 day and 1.2 x 10^5 CFU/g 5 days after the final treatment.

In the other pepper trials the total counts 5-7 days after the final treatment ranged from 5.0 x 10^3 to 4.1 x 10^4 CFU/g fresh weight.

In the study report a comparison of the data for tomato and peppers is made - no significant difference in the amount of residue on tomatoes or peppers was found. The overall mean and median counts for both commodities were well below the standard of 1 x 10^5 CFU/g fresh weight.

Table MMA 6.2.2-7: Colony forming units (CFU/g) following three applications of XenTari® WG on glasshouse grown tomatoes – 24 hour incubation on MYP- Agar

Trial	Commodity/ Variety	Sample	Mean CFU/g fresh weight	PHI (days)

LG-G-15-FG-I-01-AUG-01	Tomato/ Mekano	Control	35	0
		Control	0	3
		Treated	1.6×10^4	0
		Treated	1.9×10^4	1
		Treated	3.3×10^4	3
		Treated	1.1×10^4	7
LG-G-15-FG-I-01-AUG-02	Tomato/ Dolcella	Control	1682	0
		Control	499	3
		Treated	3.9×10^4	0
		Treated	3.9×10^4	1
		Treated	3.3×10^4	3
		Treated	2.1×10^4	7
LG-G-15-FG-I-01-BON-01	Tomato/ Lyterno RZ	Control	15	0
		Control	28	3
		Treated	3.8×10^4	0
		Treated	2.9×10^4	1
		Treated	1.7×10^4	3
		Treated	1.8×10^4	5
LG-G-15-FG-I-01-FRE-01	Tomato/ Agro	Control	154	0
		Control	107	3
		Treated	9014	0
		Treated	9686	1
		Treated	1.7×10^4	3
		Treated	1.0×10^4	5
LG-G-15-FG-I-01-WET-01 Hessen, GER-MANY	Tomato/ Cristal	Control	22	0
		Control	32	3
		Treated	6.0×10^4	0
		Treated	5.3×10^4	1
		Treated	6.2×10^4	3
		Treated	4.9×10^4	5

Table MMA 6.2.2-8: Colony forming units (CFU/g) following three applications of XenTari® WG on glasshouse grown peppers – 24 hour incubation on MYP- Agar

Trial	**Commodity/ Variety**	**Sample**	**Mean CFU/g fresh weight**	**PHI (days)**
LG-G-15-FG-I-	Pepper/	Control	115	0

Trial	Commodity/ Variety	Sample	Mean CFU/g fresh weight	PHI (days)
02-AUG-01 Baden-Württemberg, GERMANY	Jaguar	Control	0	3
		Treated	1.3×10^4	0
		Treated	1.1×10^4	1
		Treated	8405	3
		Treated	4967	7
LG-G-15-FG-I-02-AUG-02	Pepper/ unknown	Control	1058	0
		Control	604	3
		Treated	5067	0
		Treated	5392	1
		Treated	4976	3
		Treated	6230	5
LG-G-15-FG-I-02-AUG-03	Pepper/ unknown	Control	16	0
		Control	19	3
		Treated	4926	0
		Treated	1.0×10^4	1
		Treated	4215	3
		Treated	5607	5
LG-G-15-FG-I-02-BON-01	Pepper/ Davos RZ	Control	8	0
		Control	196	3
		Treated	2.8×10^4	0
		Treated	2.6×10^4	1
		Treated	2.5×10^4	3
		Treated	2.2×10^4	5
LG-G-15-FG-I-02-FRE-01	Pepper/ Amarosa	Control	97	0
		Control	17	3
		Treated	6.5×10^4	0
		Treated	5.6×10^4	1
		Treated	5.6×10^4	3
		Treated	4.1×10^4	5

Trial	Commodity/ Variety	Sample	Mean CFU/g fresh weight	PHI (days)
LG-G-15-FG-I-02-HAM-01	Pepper/ Redline	Control	25	0
		Control	57	3
		Treated	9.0×10^4	0
		Treated	1.1×10^5	1
		Treated	7.0×10^4	3
		Treated	1.2×10^5	5
LG-G-15-FG-I-02-WET-01	Pepper/ Maratos	Control	19	0
		Control	14	3
		Treated	4.6×10^4	0
		Treated	5.4×10^4	1
		Treated	2.4×10^4	3
		Treated	3.0×10^4	5

Note RMS: The QPS list (see Scientific Opinion 2017*) considered the notification of Bta ABTS 1857 as not appropriate for QPS until the respective dossier (including the literature review) is received. However, the arguments above which are based on the updated dossier will exclude the risk for consumers of the strain for pest control in agricultural settings.

* Scientific Opinion on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA. Scientific Opinion, EFSA Journal 2017; 15(3); 4664.

B.7.3 Summary and evaluation of residue behaviour

Bacillus thuringiensis subsp. *aizawai* is a gram-positive, spore forming, rod-shaped bacterium that forms a characteristic crystalline protein inclusion. *Bacillus thuringiensis* subsp. *aizawai* is toxic to larvae of some Lepidopteran insects upon ingestion.

The representative uses supported in this dossier are for outdoor and protected peppers.

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 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. 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.

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.

B.7.4 References relied on

See B.6 MA for summary literature search.

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KMA6.1 /01	Konecka E, Baranek J, Bielińska I, Tadeja A, Kaznowski A	2014	Persistence of the spores of *B. thuringiensis* subsp. *kurstaki* from Foray bioinsecticide in gleysol and on leaves. Journal of Science of the Total Environment (2014), Vol. 472, pp 296-301. Not GLP Published	N	N	N/A	N/A
KMA6.1 /02	Frederiksen, K, Rosenquist, H, Jørgensen, K, Wilcks, A.	2006	Occurrence of Natural *Bacillus thuringiensis* Contaminants and Residues of *Bacillus thuringiensis*-Based Insecticides on Fresh Fruits and Vegetables. Journal of Applied and Environmental Microbiology (May 2006), Vol. 72, No.5, pp 3435-3440. Not GLP Published	N	N	N/A	N/A
KMA6.1 /03	Choma, C, Guinebretiére, MH, Carlin, F, Schmitt, P, Velge, P,	2000	Prevalence, characterisation and growth of *Bacillus cereus* in commercial cooked chilled foods containing vegetables. Journal of Applied Microbiology 2000, 88: 617-625. Not GLP	N	N	N/A	N/A

	Garnum, PE. & Nguyen-The, C.		Published				
KMA6.1/04	Colla, F	2010	Study of *Bacillus thuringiensis* behaviour in food environment by genome-wide transcriptome analysis. University of Verona. Not GLP Published	N	N	N/A	N/A
KMA6.1/06	Zhou, G, Yan, J, Dasheng, Z, Zhou, X, Yuan, Z.	2008	The residual occurrences of *Bacillus thuringiensis* bio pesticides in food and beverages. International Journal of Food Microbiology 127 (2008) 68-72. Not GLP Published	N	N	N/A	N/A
KMA6.2.2/ 01	Pedersen, Damgard and Eilenberg	1995	Dispersal of *Bacillus thuringiensis var. kurstaki* in an experimental cabbage field. Canadian. Journal of Microbiology. 41 (2) 118-125) Not GLP Published **PREVIOUSLY EVALUATED IN BTK DAR DENMARK (2008)**	N	N	N/A	N/A
KMA6.2.2/02	Haddad, M, Polanczyk, R, Alves, S & de Olivera Garica, M	2005	Field Persistence of *Bacillus thuringiensis* on maize leaves. Brazilian Journal of Microbiology, 36: 309-314 Not GLP Published	N	N	N/A	N/A
KMA6.2.2/03	Stephan D;	2013a	Investigation of the spore concentration of *Bacillus thuringiensis* on marketable Salanova Lettuce Heads after application with XenTari® WG	N	N	N/A	N/A

			Julius Kühn -Institut Federal Research Institute for Cultivated Plants Institute for Biological Plant Protection Not GLP Not Published				
KMA6.2 .2/04	Stephan D	2013b	Summary of investigations on residues of *Bacillus thuringiensis* on lettuce and tomato. Julius Kühn -Institut Federal Research Institute for Cultivated Plants Institute for Biological Plant Protection Not GLP Not Published	N	N	N/A	N/A
KMA6.2 .2/05	Ehrhardt J	2016	Residue study (decline) on tomato following three foliar applications of XenTari® WG under protected conditions in Germany 2015. Versuchswesen Pflanzenschutz Report No: VP15-1-74 GLP Unpublished	N	Y	New data submitted for the first time	VBC
KMA6.2 .2/06	Wagner A	2016	Trials of Residue on Tomatoes and Peppers, Part of the Residues Research by the Minor Uses Working group. Julius Kühn -Institut Federal Research Institute for Cultivated Plants Institute for Biological Plant Protection GLP Unpublished	N	N	N/A	N/A