Renewal
Assessment Report

Bacillus thuringiensis ssp. aizawai strain ABTS-1857

Volume 3 – B.6 Effects on human health

Rapporteur Member State: The Netherlands

Co-Rapporteur Member State: Germany

Version history

When	What
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B Summary of the data and information

B.6 Effects on human health

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.6.1 Tier I

B.6.1.1 Basic information

Bacillus thuringiensis is a ubiquitous Gram-positive spore-forming bacterium that synthesises crystalline proteinaceous inclusions containing δ-endotoxins (Crystal (Cry) and cytolytic (Cyt) proteins), some of which have insecticidal activity. The species B. thuringiensis is characterised by the production of crystal inclusions in parallel with spore formation (EFSA 2016). Following ingestion by the target insect species the crystals are solubilised in the midgut, activated by protease enzymes and bind to specific cell membrane receptors, leading to cell disruption, infection and death. 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. The insecticidal Cry toxins constitute 20-30% of the cell dry weight; synthesis of the toxins is controlled by a number of coordinating mechanisms at the transcriptional, post-transcriptional and post-translational levels.

The pathogenicity of Bt to insects involves targeting specific cadherin receptors in the host; indicating that the mode of action involves effects on host-specific cell adhesion proteins. These toxins are insect-specific, are inactivated by protease enzymes present in the human gastrointestinal tract and do not affect the mammalian gastrointestinal tract (Shimada *et al*., 2006; Berlitz, 2006).

Bacillus thuringiensis is closely related to the occasional human pathogen *Bacillus cereus.* Some strains of *B. cereus* cause human food poisoning due to the contamination of food with bacterial

spores, the subsequent germination of spores of pathogenic strains of *B. cereus* in improperly stored food and the production of a number of enterotoxins. 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[1]. (summary?) Other work by the same authors[2] (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. While no differences were seen in the survival of cells of different strains of *B. cereus*, marked differences were seen in sporulation tendency. The authors therefore suggest that this may be an important factor in determining the potential for food poisoning.

Ingestion of *B. cereus*-contaminated food results in two distinct types of illness, namely diarrhoeal and emetic syndromes.

The DNA of *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857) has been analysed by amplified fragment length polymorphism (AFLP), producing a visual DNA fingerprint[3]. Comparison to data obtained from over 300 strains of *B. thuringiensis* and *B. cereus* have been used to construct a phylogenetic tree which shows that the strains could be placed into 3 clusters, each containing 3 or 4 branches. Using this technique, *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857) has been placed into Cluster 1 Branch C. This phylogenetic grouping consists of many *Bacillus thuringiensis* strains that are not toxigenic to vertebrates, and is distinctly separate from pathogenic and toxigenic *Bacillus* isolates, which are included in a different cluster. Genomic comparison of *B. cereus* and *B. thuringiensis* strains also reveals differences in terms of virulence, metabolic competence, structural components and regulatory mechanisms. Genome analysis provides insight into the evolutionary relationships among these species, as well as the molecular mechanisms contributing to their host range and virulence. The genome analysis presented for *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857) clearly indicates clustering away from known pathogenic *B. cereus* strains.

The Bacillus cereus group, also known as *B. cereus sensu lato*, is a subdivision of the Bacillus genus and consists of several species, including *B. cereus sensu stricto* and *B. thuringiensis*. The current taxonomy of the B. cereus group and the status of separate species mainly rely on phenotypic characteristics. *Bacillus cereus* and *B. thuringiensis* strains are usually not discriminated in clinical diagnostics or food microbiology.

B. cereus and *B. thuringiensis* strains are very similar and, consequently, are not usually distinguished in cases of food poisoning using routine methodology. The EFSA Panel on Biological Hazards (BIOHAZ)[4] 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). *B. cereus* diarrhoeal syndrome has a typical incubation time of 8-16 hours and is associated with gastrointestinal pain and watery diarrhoea, and is attributed to the action of the enterotoxins haemolysin BL (hBL), non-haemolytic enterotoxin (Nhe) and cytotoxin K (CytK). Enterotoxin is degraded by heating and by stomach enzymes (Ceuppens et al. (2012)); its action may therefore be due to

[1]Ceuppens S *et al*., (2012). Enterotoxin production by *Bacillus cereus* under gastrointestinal conditions and their immunological detection by commercially available kits. Foodborne Pathogens Disease 9(12):1130-1136.

[2]Ceuppens S *et al*., (2012). Inactivation of *Bacillus cereus* vegetative cells by gastric acid and bile during *in vitro* gastrointestinal transit. Gut Pathogens 4(11):

[3]Benson T (2005). Genetic comparison of *Bacillus thuringiensis* ssp. *kurstaki strain* HD-1 to other *Bacillus* strains using AFLP. Valent BioSciences Corporation.

[4]EFSA Panel on Biological Hazards (BIOHAZ). 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):4524-4516.

toxin production in the gastrointestinal tract. *B. cereus* emetic syndrome is associated with nausea and vomiting and has a typical incubation time of 1-5 hours. Symptoms are caused by the heat-stable toxin cereulide (*ces*). Carlin *et al*. (2006)[5] selected 100 *B. cereus* strains to include clinical isolates from food remnants connected to both emetic and diarrheal foodborne outbreaks and isolates from food and the environment. Expression of the *ces* gene (restricted to emetic B. cereus see Ehling-Schulz, M. et al. (2006)) was analysed and shown to vary more than 1000-fold between strains (<1ng to 1600 ng). So far the cereulide toxin synthesis (ces) gene has not been found in B. thuringiensis (EFSA 2016). In addition, Lücking, G. et al. (2002) demonstrated that cereulide synthesis in emetic Bacillus cereus is controlled by the transition state regulator AbrB, but not by the virulence regulator PlcR

It is generally considered that food poisoning may result from contamination with *Bacillus cereus* at levels of 10^5 organisms/g food; however this will vary between strains and cases of emetic and diarrhoeal syndromes have been reported at lower levels of contamination. The EFSA BIOHAZ document (EFSA, 2016) notes that *B. cereus* levels as low as 10^5 organisms/g food have been implicated in food poisoning.

EFSA (2005)[6] indicates that the strains of *B. cereus* causing diarrhoea are difficult to identify as the mechanisms leading to symptoms are complex. It is further acknowledged that not all *B. cereus* strains are food-borne pathogens; the more recent EFSA BIOHAZ document noting that there is no definitive evidence for the role of these toxins (alone or in combination) in the diarrhoeal syndrome.

In a detailed survey of the occurrence of *B. cereus*-like bacteria in food, Rosenquist *et al*. (2005)[7], discussed in the original DAR, highlights the ubiquitous nature and natural occurrence of *Bacillus cereus*. Detailed analysis in this study indicated that only 35.7 % of these strains produced crystals and were positive for *cry1* genes, indicating that the strain originated from a *B. thuringiensis* spray treatment. Of these ten isolates, four were from sausage, pasta, bread and honey; foods not normally treated with Bt insecticides. Only six (15%) of the original 40 isolates selected for more detailed taxonomic analysis could have possibly have originated from Bt sprays; however these six isolates were from red pepper, cauliflower, leeks, salad and figs, none of which are typically associated with food poisoning caused by *B. cereus*. No evidence was provided (nor was it implied) that any of the strains of *B. thuringiensis* or *B. cereus* were involved in any cases or outbreaks of food poisoning. This report highlights the innocuousness of most *B. cereus* strains, which are naturally found in a large number of foods and may be ingested by humans without significant consequence.

Based on the EFSA BIOHAZ document RMS concluded that neither cereulide, the emetic toxin of B. cereus, nor the highly cytotoxic form of CytK, namely CytK1, are produced by Bt. CytK2, however is not considered to be involved in enterotoxicity of B. cereus group strains (see Fagerlund et al. (2004)). All other enterotoxins such as Non-hemolytic enterotoxin (NHE) or Hemolysin BL (HBL) or PlcR (could be potentially produced by members of Bt. Other virulence factors such as sphingomyelase or Haemolysin II or InhA1 or NprA have so far not been detected in Bt. 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.

[5]Carlin F *et al*. (2006). Emetic toxin-producing strains of *Bacillus cereus* show distinct characteristics within the *Bacillus cereus* group. International Journal of Food Microbiology 109(1-2):132-138.

[6]Opinion of the Scientific Panel on Biological Hazards on Bacillus cereus and other Bacillus spp in foodstuffs. EFSA Journal 175: 1-48.

[7]Rosenquist H *et al*. (2005). Occurrence and significance of *Bacillus cereus* and *Bacillus thuringiensis* in ready-to-eat food. FEMS Microbiology Letters 250(1):129-136.

So, nearly all *B. cereus* strains contain the non-haemolytic enterotoxin complex (*nhe*) genes, while haemolysin BL complex (*hbl*) and cytotoxin K (*cytK*) are detected in about 30-70% of isolates (EFSA BIOHAZ document). The enterotoxigenic profiles of 51 B. cereus food-related strains were compared to those of 37 B. cereus food-poisoning strains. cytK and association of hbl-nhe-cytK enterotoxin genes were more frequent among diarrheal strains (73 and 63%) than among food-borne strains (37 and 33%). Unlike diar-rheal strains, food-borne strains showed frequent nhe and hbl gene polymorphisms and were often low toxin producers (Guinebretière, M-H. et al. (2002)). Böhm et al. (2016) worked on genetic sequence and differences in cytotoxicity due to the promoters. The unusually long promoter regions of nhe and hbl might be important for a concomitant interaction of several global regulators. To gain insight into the origin of enterotoxin expression heterogeneity in different strains, the architecture and role of 50 intergenic regions (50 IGRs) upstream of the nhe and hbl operons was investigated. Electrophoretic mobility shift assays showed that the branched-chain amino acid sensing regulator CodY binds to both nhe and hbl 50 UTR downstream of the promoter, potentially acting as a nutrient-responsive roadblock repressor of toxin gene transcription. The nutrient-sensitive transcriptional regulator CodY is far less conserved, perhaps conferring varying strengths of CodY binding, which might modulate toxin synthesis in a strain-specific manner. PlcR binding sites are highly conserved among all B. cereus sensu lato strains, indicating that this regulator does not significantly contribute to the heterogeneity in virulence potentials.

These genes are also present in *B. thuringiensis* strains; however the majority of *B. thuringiensis* strains investigated have been shown not to produce enterotoxins. *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857) has been shown not to produce β-exotoxin, cytolytic proteins or enterotoxins during the production process

The level of enterotoxin production by different *B. cereus* strains is also highly variable[8], and it has been suggested[9] that only a small number of strains are able to produce sufficient toxin to cause diarrhoeal symptoms. Using the strains characterized within the EU research project 'Preventing *Bacillus cereus* foodborne poisoning in Europe' (QLK1-CT-2001-00854), Moravek *et al*. (2006)[10] showed that the amount of enterotoxins produced showed a very high level of variation. The authors therefore questioned whether the detection of enterotoxin genes in *B. cereus* by PCR or an absence/presence test for toxin production is an appropriate method to estimate the virulence of *B. cereus* isolates.

One report (Damgaard, 1995)[11] shows the production of enterotoxin by *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857) but only at relatively low levels and under very specific culture conditions. Culture of this strain in Brain Heart Infusion Broth (BHIB) resulted in an enterotoxin titre of enterotoxin of 23, compared to 1629 in an enterotoxin-producing *B. cereus* strain. It is possible, therefore, that low levels of enterotoxin may be produced by *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857) under very specific conditions; however BHIB is shown to induce the production of toxins not normally expressed, as demonstrated by Beattie & Williams (1999)[12], who showed differential toxicity for *B. cereus* grown in BHIB and milk. *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857) has been shown not to produce enterotoxins under the conditions of manufacture. These condi-

[8]Dufrenne *et al*. (1995). Characteristics of some psychotropic *Bacillus cereus* isolates. International Journal of Food Microbiology 27(2-3): 175-183.

[9]Ronner & Andersen (1995). Emerging Microbial Risks in Processes Case: How serious is *Bacillus cereus*?

[10]Moravek *et al*. (2006). Determination of the toxic potential of Bacillus cereus isolates by quantitative enterotoxin analyses. FEMS Microbiology Letters 257(2):293-8.

[11]Damgaard PH (1995). Diarrhoeal enterotoxin production by strains of *Bacillus thuringiensis* isolated from commercial *Bacillus thuringiensis-based* insecticides. FEMS Immunology & Medical Microbiology 12:245-250.

[12]Beattie SH & Williams AG (1999). Detection of toxigenic strains of *Bacillus cereus* and other *Bacillus* spp. with an improved cytotoxicity assay. Letters in Applied Microbiology 28(3):221-225.

tions are carefully designed to be optimal for growth under which the production of enterotoxin would be most likely (as is seen for rapidly growing *B. cereus*). Even when a gene is shown to be present, the required operons may be absent and genes may not be expressed, or only at very low levels.

It can be concluded, therefore, that the production of enterotoxin by *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857) would be unlikely to occur in practice and, even if the enterotoxin was produced, the low levels represent a significant safety margin (70x) over the levels of *B. cereus* (10^5 organisms/g food) typically required to cause food poisoning.

The emetic toxin cereulide is produced by several but not all strains of *B. cereus* and there are no indication that it is produced by any strain of *B. thuringiensis*. Mikami *et al*. (1995)[13] showed the production of emetic toxin by some of 524 strains of *B. cereus* but none of 90 strains of *B. thuringiensis* examined.

Harriam (2015), also characterized Enterotoxigenic Bacillus cereus and Bacillus thuringiensis Spores in U.S. retail Spices by using a novel chromogenic agar, B. cereus spores were isolated from 77 samples (31%) and B. thuringiensis spores were isolated from 11 (4%) samples. Levels of B. cereus ranged from <3 MPN/g to 1600 MPN/g. B. thuringiensis ranged from 3.6 to 240 MPN/g with only one sample in the 100-1600 range - the rest were evenly distributed between ranges 1 to 10 and 10 to 100. Eighty four of the 88 spices tested possessed either one of the enterotoxin genes. None of the isolates tested positive for the emetic toxin (ces) gene.

Conclusion related to food poisoning

B. thuringiensis strains cannot produce emetic toxin (cereulide) as the genome does not include this gene. Some strains of *B. cereus* produce diarrhoeal enterotoxins; however the level of toxin production is highly variable and strain-specific. The production of Hbl and Nhe enterotoxins is governed by a number of factors including growth rate and various environmental or nutritional factors; however bacterial numbers and the pleiotropic gene regulator PlcR are shown to be key activator of enterotoxin production[14].

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 (Ceuppens et al (2012)). There are several studies indicating that some Bt strains will germinate in the human-flora-associated rats. Germination, but not growth was observed in at least two Bt strains. In the study by Wilcks et al (2006), the density of the two commercial Bt strains, one of which was a Btk strain (DMU67R) and the other one a Bti strain (HD567), was assessed in intestine samples and faeces upon oral gavage of human-flora-associated rats for 4 days. In animals fed spores, *B. thuringiensis* cells were detected in faecal and intestinal samples of all animals, 1 day after the last administration. Vegetative cells of Btk poorly survived the gastric passage, whereas Bti vegetative cells did not survive gastric passage. In 5/6 animals, Btk but not Bti, was detectable two weeks post administration in faeces samples, when feeding the animals the spores. One specific animal differed from the rest by a two log higher density of Btk DMU67R cells in the faecal samples (10^4 CFU/g) being observed at the end of the experiment. A similar tendency was observed in intestine samples. Btk but not Bti was still detectable in the samples two weeks after the last dosage and the animal having the high counts in faeces also had significant higher CFU numbers in intestine samples. Heat treatment of intestinal samples of this animal, thereby killing the vegetative cells but not the

[13]Mikami *et al*. (1995). Examination of toxin production from environmental *Bacillus cereus* and *Bacillus thuringiensis*. Yakugaku Zasshi 115(9):742-748.

[14]Ceuppens S *et al*. (2011). Regulation of toxin production by *Bacillus cereus* and its food safety implications. Critical Reviews in Microbiology 37(3):188-213.

spores, revealed the presence of a high percentage of living vegetative cells. The same was observed in samples from 3 animals fed Bti untreated spores at sacrifice on day 5 post treatment. It can be concluded that vegetative cells and spores can survive the gastric passage, but the rate and extend is dependant of the Bt strain. Moreover, spores that survive the gastric passage do germinate in the gut. No enterotoxin production was shown and there were no effects on the composition of the faecal biota. The study did not reveal any health issues potentially associated with either of the two commercial strains investigated.

However, and while these conditions of enterotoxin production are encountered in the insect gut they are not present in the mammalian gastrointestinal tract. Furthermore, commercial Bt strains have been demonstrated to grow significantly slower than pathogenic (or even non-pathogenic) strains of *B. cereus* (Hanssen et al. (2011)). Bacterial enterotoxins are shown not to specifically bind to mammalian intestinal cells (reference?) and do not affect their function.

The hemolytic enterotoxin hemolysin BL (HBL), the non-hemolytic enterotoxin (Nhe), and cytotoxin K (CytK) are claimed to play a major role in diarrheal disease. However, with the exception of HBL (rabbit ilealloops), a direct involvement of these toxins in diarrhea has not been clarified by the use of suitable animal models. However, with the exception of HBL (Section 2), a direct involvement of these toxins in diarrhea has not been clarified by the use of suitable animal models.(Senesi & Ghelardi. 2010). HBL genes are present in about 45–65% of B. cereus strains. (Thaenthanee et al.; Dietrich et al. 1999) electron microscopy has shown pore formation from toxins in E.coli, etc. but no such data has been found for B.c. diarrheal enterotoxins.

B. thuringiensis also shows critical differences to pathogenic *B. cereus* strains in the S-layer a factor which governs bacterial cell adhesion to cells lining the gastrointestinal tract, a critical determinant of pathogenicity.

In summary, therefore, 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.

This conclusion is based on a number of factors, including:

- The absence of genes for emetic toxin, shown by genome sequencing
- Critical differences between this strain and B. cereus relating to the expression of enterotoxin genes
- An absence of reports of food poisoning outbreaks associated with the expression and presence of this strain, despite widespread and long-term (>40 years) commercial use on food crops

According to the RMS the following factors can be added (in case all references are submitted):

- Commercial Bt strains, including Bta ABTS 1857, underwent extensive pathogenicity testing, confirming that the strains are not toxic, pathogenic or infective upon either route of exposure (please refer to info provided in MA Section 5 and MP Section 8)
- For all commercial Bt strains, including Bta ABTS 1857, methods are currently available for unequivocal identification of the strain distinguishing them from other Bacillus sp. including B. cereus and even from other Btk strains (please refer to MA, Section 1

- Bt in general and this particularly applies to commercial Bt strains, which were selected for insecticidal activity, are first and foremost insect pathogens. As a result, insecticidal Bt strains are better adapted to complete their life cycle in infested host insects but not in other environmental compartment including the human intestine
- Commercial Bt strains, including Bta ABTS 1857 differ from pathogenic B. cereus strains with regard to their physiology and their ability to act as a pathogen:
  1) They 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)
  2) Insecticidal Bt strains, being highly adapted to their insect hosts and requiring specific conditions for spore outgrowth, are unlikely to survive the gastrointestinal passage, germinate and multiply in the human intestine.
  3) They do not adhere to and invade the intestinal wall
  4) They do not produce toxins in the host at relevant levels

In the public literature RMS found a recent paper titled "In defence of Bacillus thuringiensis, the safest and most successful microbial insecticide available to humanity—a response to EFSA (2016)" written by Ben Raymond (FEMS Microbiology Ecology, 93, 2017, fix084).
RMS agrees with the conclusion of the paper that the recent controversial case of food poisoning in Germany presents no convincing evidence that Bt was the causative agent, since individuals with food poisoning had also consumed a dose of Bc sufficient to cause the observed level of infection. Overall, the MLST databases, the epidemiological studies and safety testing literature present a well-informed and coherent view of the biology and ecology of the Bc group. The arguments in the EFSA report, that we do not understand the risks of consuming Bt spores, are therefore unfounded and overly cautious. An analysis of studies cited in EFSA's opinion used to question Bt safety (Rosenquist et al. 2005, Frederiksen et al. 2006) show not only do humans routinely eat high levels of this species, but that most of the strains (>80%) consumed are naturally occurring, not from biopesticides. Yet even at rates not considered acceptable under Danish guidelines, there is no evidence that consumption has ever resulted in food poisoning. Furthermore, strains of entomocidal Bt are not capable of infecting vertebrates at extremely high doses in controlled laboratory tests and there are no robust data to suggest that humans might be an exception. Phylogenetic analyses of ecological differentiation across the Bc group suggest that there are very few strains of Bt with elevated risks for vertebrates (Guinebreti `ere et al. 2010; Raymond et al. 2010b; Raymond and Bonsall 2013). This would include the subsp. konkukian, which was originally isolated from a soldier severely injured by a land mine (Hernan-dez, Ramisse and Ducoureau 1998). That isolate did indeed pose a greater risk to mice than biopesticidal strains of Bt (Hernandez et al. 2000). Crucially, the Bt konkukian can be firmly placed in the anthracis clade and is distantly related to all the biopesticidal strains (Han et al. 2006; Raymond et al. 2010b; Raymond and Bonsall 2013); it is also not demonstrably pathogenic to insects. Based on the ecological differentiation across the Bc group, we would not recommend licensing any Bt products that show a similar biological affinity to B. anthracis.

Here below, the abstract is presented:
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. Moreover, 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 pos-

ing 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.

According to SANCO/10754/2005 rev.5, 2005, an assessment for a certain strain can be used for another strain only when there is sufficient evidence that the strains do not differ with regard to properties of potential relevance for human health. This is obviously not the case for commercial Bt and pathogenic B. cereus strains, as they do significantly differ in their toxigenic potential, but also in their physiology and their environmental behaviour. The low toxigenic potential of Bta ABTS 1857 together with the proven absence of pathogenicity of the strain indicates that the risk for consumers following use of Bta ABTS 1857 for pest control in agricultural settings is acceptable.

Summary and abstracts of cited literature:

Report:	MMA 5.1/01, Shimada N, Miyamoto K, Kanda K & Murata H (2006)
Title:	*Bacillus thuringiensis* insecticidal Cry1Ab toxin does not affect the membrane integrity of the mammalian intestinal epithelial cells: An *in vitro* study.
Reference:	In Vitro Cellular & Developmental Biology Animal 42 (1-2): 45-49
Guidelines:	None
GLP:	No

Abstract: The affinity of Cry1Ab to the cellular brush border membrane vesicles (BBMV) of two mammalian intestinal cells and its effects on the membrane potential of three mammalian intestinal cells were compared to its effects on the silkworm midgut cell. Cry1Ab was found to bind very weakly to the bovine and porcine BBMV compared to the silkworm BBMV. Silkworm cells developed severe membrane potential changes within 1 hour of exposure; whereas no such changes were observed in bovine, porcine or human intestinal cells. The study therefore suggests that, although Cry1Ab toxin may bind weakly or non-specifically to certain BBMV components in the mammalian intestinal cell, it does not damage the cell membrane and consequently has no adverse effects on the cell.

Material: Cry1Ab toxin, purified from a recombinant *E. coli* strain transfected with the *crylAb* gene of *B. thuringiensis* ssp. *kurstaki* (strain HD-1) and activated to produce the CrylAb toxin.

Methods: The extent of binding of Cry1Ab to purified bovine and porcine small intestine BBMV was assessed using a co-precipitation assay and BIAcore assay. The co-precipitation assay involved incubation of Cry1Ab with BBMV followed by centrifugation, SDS-PAGE and immunoblotting with anti-Cry1Ab antibody. The BIAcore assay measured the extent of binding of Cry1Ab to immobilised BBMV on a sensor chip, using an optical biosensor. The membrane potential effects of Cry1Ab were measured in bovine and porcine small intestine cells and in a human intestinal epithelial (HIE) cell line, using the voltage-dependent fluorescent dye DiBAC4.

Findings: In the co-precipitation assay, CrylAb bound to the bovine and porcine BBMV less strongly than to silkworm BBMV. Although there was indication of binding to the mammalian cell BBMV, this binding was indicated by the BIAcore assay to have weak affinity. In contrast to effects on the membrane potential of silkworm intestinal cells, no effects were seen in bovine or porcine cells or in the HIE cell line. No effect of Cry1Ab was seen on mammalian cell viability (assessed using LDH release assay), even following the longest incubation of 48 hours.

Conclusion: The study suggests that, although Cry1Ab toxin may bind weakly or non-specifically to certain BBMV components in the mammalian intestinal cell, it does not damage the cell membrane and consequently has no adverse effects on the cell.

Report:	MMA 5.1/02, Berlitz DL (2006)
Title:	Toxicology effects of δ-endotoxins and β-exotoxins of *Bacillus thuringiensis* in

	Wistar rats.
Reference:	Neotropical Biology and Conservation 1(1):35-38
Guidelines:	None
GLP:	No

Abstract: The authors investigated the fate of *Bacillus thuringiensis thuringiensis* and *Bacillus thuringiensis* ssp. *aizawai* toxins following oral administration to Wistar rats. SDS-PAGE was used to profile protein toxins in the faeces at various time points (12-36 hours) following gavage administration. Data showed that the toxin proteins were digested by the actions of the stomach.

Material: XenTari® commercial product containing *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857). *Bacillus thuringiensis thuringiensis*.

Methods: Male adult Wistar rats (80-100 days old) were gavaged with the test material at 0, 12 and 24 hours. Total faecal collections were made at 12, 24 and 36 hours following treatment. The rats were killed at 24 or 36 hours after application and the stomach assessed microscopically. Protein content of the faecal samples and stomach content was investigated using SDS-PAGE using δ-endotoxins isolated from this bacterial strain as a standard.

Findings: No microscopic effects of treatment were observed on the stomach. SDS-PAGE demonstrated the digestion of endotoxin into smaller peptide fragments by the actions of the stomach.

Conclusion: The results of this study demonstrate that *Bacillus thuringiensis thuringiensis* and *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857) endotoxins are digested in the mammalian gastrointestinal tract.

Report:	MMA 5.1/03; Wilcks A, Hansen BM, Hendricksen NB & Licht TR (2006).
Title:	Persistence of *Bacillus thuringiensis* bioinsecticides in the gut of human-flora-associated rats.
Reference:	FEMS Immunology & Medical Microbiology 48:410-418.
Guidelines:	None
GLP:	No

Abstract: The capability of two bioinsecticide strains of *Bacillus thuringiensis* (ssp. *israelensis* and ssp. *kurstaki)* to germinate and persist in the gastrointestinal tract was studied in human-flora-associated rats. Rats were dosed either with vegetative cells or spores of the bacteria for 4 consecutive days. In animals fed spores, *B. thuringiensis* cells were detected in faecal and intestinal samples of all animals, whereas vegetative cells survived poorly. Heat-treatment of intestinal samples which kills vegetative cells, revealed that *B. thuringiensis* spores were capable of germination in the gastrointestinal tract. In one animal fed spores of *B. thuringiensis* ssp. *kurstaki*, these bacteria were detected at a high density in faecal and intestinal samples, even two weeks after the last dose. In the same animal, translocation of *B. thuringiensis* ssp. *kurstaki* to the

spleen was observed; however, no other adverse effects were apparent. Denaturing gradient gel electrophoresis of PCR-amplified bacterial 16S rRNA genes in faecal samples revealed no major effect of *B. thuringiensis* on the composition of the indigenous gut bacteria. Additionally, no cytotoxic effect was detectable in gut samples by Vero cell assay.

Materials: *Bacillus thuringiensis* ssp. kurstaki DMU67R (identical to HD1); *B. thuringiensis* ssp. *israelensis* HD567.

Methods: Groups of six human-flora-associated (HFA) SD rats (7-9 weeks old) were administered oral doses of 1 mL of bacterial preparations on four consecutive days. Rats fed *B. thuringiensis* ssp. *kurstak*i DMU67R received either 10^7 spores (untreated or heat-treated) or 10^7-10^8 vegetative cells per day. Animals dosed with *B. thuringiensis* ssp. *israelensis* HD567 received 10^8 untreated spores, 10^6 heat-treated spores, or 10^8 vegetative cells. Half of the animals were sacrificed at Day 5 and the remaining half at Day 18. Intestinal and faecal samples were analysed and bacteria enumerated. The spleen and liver were removed from the animals at Day 18. *B. cereus*-like bacteria, *B. thuringiensis* ssp. *kurstaki* DMU67R and *B. thuringiensis* ssp. *israelensis* HD567; lactobacilli; coliforms and enterococci; total aerobic and anaerobic counts were enumerated in the faecal and intestinal samples. The *B. thuringiensis* strains were also enumerated in spleen and liver samples. Intestinal samples were heat-treated to kill the *B. thuringiensis* vegetative cells, followed by enumeration of the spores. DNA was extracted from the faecal samples and subjected to PCR. The PCR product was assessed using denaturing gradient gel electrophoresis (DGGE) and prominent bands sequenced. Intestinal samples were assessed for enterotoxin production using the Vero cell assay and a toxin detection kit.

Findings: No *B. cereus*-like cells were detected in faecal samples prior to treatment, or following treatment with irradiated spores of either strain. Dosing with vegetative cells resulted in detectable cells in rats fed *B. thuringiensis* ssp. *kurstaki* DMU67R, and on the days of dosing only. In experiments with *B. thuringiensis* ssp. *kurstaki* DMU67R, no differences in the numbers of *B. thuringiensis* cells recovered from faecal samples were seen between rats administered untreated and heat-treated (to mimic cooking food) spores. In 5/6 animals fed spores, Btk but not Bti, was detectable 2 weeks post administration. On Day 5, *B. thuringiensis* ssp. *kurstaki* DMU67R was detectable in the intestinal samples of all rats administered this strain (either as untreated or heat-treated spores or vegetative spores); at two weeks following administration, cells were only detected in the intestinal samples of rats fed live (untreated or heat-treated) spores. Much higher cell numbers were observed in one rat, and translocation to the spleen (but not the liver) was seen in this animal.

Table 6.1.1-1 Presence of Btk DMU67R in intestinal samples from HFA rats, dosed either with untreated spores, heat-treated spores, or vegetative cells.

	Density of DMU67R (log CFU g^{-1} intestinal content)					
	Day 5			Day 18		
Intestinal sample	Untreated spores	Heat-treated spores	Vegetative cells	Untreated spores	Heat-treated spores	Vegetative cells
Duodenum	2.49 ± 0.47	2.64 (1/3)*	†	†	2.53 ± 1.31 (2/3)	†
Ileum	3.32 ± 0.25	2.60 ± 0.96	†	†	3.39 (1/3)	†
Caecum	5.70 ± 0.12	5.31 ± 0.14	1.30 (1/3)	1.30 ± 0.00 (2/3)	2.11 ± 1.15	†
Colon	5.82 ± 0.13	5.73 ± 0.17	1.43 ± 0.38	1.30 (1/3)	2.64 ± 0.91 (2/3)	†

*Number in brackets indicates how many animals were positive out of total number of animals tested if this differs from three.
†Number of cells was below detection limit (10 CFU g^{-1} intestinal sample).

For *B. thuringiensis* ssp. *israelensis* HD567, cells were detected at Day 5 only in rats administered live spores. No cells were detected at Day 18.

Table 6.1.1-2 Presence of Bti HD567 in intestinal samples from HFA rats, dosed either with untreated spores, heat-treated spores, or vegetative cells.

	Density of HD567 (log CFU g^{-1} intestinal content)					
	Day 5			Day 18		
Intestinal sample	Untreated spores	Heat-treated spores	Vegetative cells	Untreated spores	Heat-treated spores	Vegetative cells
Duodenum	4.36 ± 0.34	1.60 (1/3)*	†	†	†	†
Ileum	5.85 ± 0.27	2.96 ± 0.59	†	†	†	†
Caecum	5.79 ± 0.06	3.47 ± 0.51	†	†	†	†
Colon	5.84 ± 0.07	3.44 ± 0.60	†	†	†	†

*Number in brackets indicates how many animals were positive out of total number of animals tested if this differs from three.
†Number of cells was below detection limit (10 CFU g^{-1} intestinal sample).

Intestinal samples from animal no. 15 fed untreated spores of Btk DMU67R were heat treated to kill bacteria in the vegetative state. This revealed that 90% of the cells found in the small gastrointestinal tract (duodenum and ileum) were present as vegetative cells, hence the spores had germinated. In the large intestinal samples (caecum and colon) the percentage of vegetative cells was lower. Similar results were obtained for animals fed spores of Bti HD567.

Administration of either strain did not reveal any significant effects on the composition of the faecal biota. Enterotoxin was not detected in intestinal samples taken from rats treated with either strain.

Conclusion: This study shows the possible growth of *B. thuringiensis strains* in the rat intestinal tract; findings suggest germination in the small intestine and sporulation in the large intestine prior to being excreted in the faeces. Heat treatment of spores, mimicking heating of food) did not affect growth in the gut. No enterotoxin production was shown and there were no effects on the composition of the faecal biota. With the exception of the finding of translocation of *B. thuringiensis* ssp. *kurstaki* to the spleen in

a single animal, the authors conclude that the study did not reveal any health issues potentially associated with either of the two commercial strains investigated.

Report KMA 1.3/07 Lücking, G. *et al.* (2002). Cereulide synthesis in emetic *Bacillus cereus* is controlled by the transition state regulator AbrB, but not by the virulence regulator PlcR. Microbiology, Vol. 155, pp. 922-931.

GLP No

Summary
Cereulide, a depsipeptide structurally related to the antibiotic valinomycin, is responsible for the emetic type of gastrointestinal disease caused by *Bacillus cereus*. Recently, it has been shown that cereulide is produced non-ribosomally by the plasmid-encoded peptide synthetase Ces. Using deletion mutants of the emetic reference strain *B. cereus* F4810/72, the influence of the well-known transcription factors PlcR, Spo0A and AbrB on cereulide production and on the transcription of the cereulide synthetase gene cluster was investigated. The data demonstrate that cereulide synthesis is independent of the *B. cereus* specific virulence regulator PlcR but belongs to the Spo0A-AbrB regulon. Although cereulide production turned out to be independent of sporulation, it required the activity of the sporulation factor Spo0A. The σ^A-promoted transcription of *spo0A* was found to be crucial for cereulide production, while the σ^H-driven transcription of *spo0A* did not affect cereulide synthesis. Overexpression of the transition state factor AbrB in *B. cereus* F4810/72 resulted in a non-toxic phenotype. Moreover, AbrB was shown to bind efficiently to the main promoter region of the *ces* operon, indicating that AbrB acts as a repressor of cereulide production by negatively affecting *ces* transcription.

Report KMA 1.3/05 Fagerlund, A. *et al.* (2004). Genetic and functional analysis of the *cytK* family of genes in *Bacillus cereus*. Microbiology, Vol. 150, pp. 2689-2697.

GLP No

Summary
CytK is a pore-forming toxin of *Bacillus cereus* that has been linked to a case of necrotic enteritis. PCR products of the expected size were generated with *cytK* primers in 13 of 29 strains. Six strains were PCR-positive for the related gene *hly-II*, which encodes haemolysin II, a protein that is 37% identical to the original CytK. Five of the strains were positive for both genes. The DNA sequences of putative *cytK* genes from three positive strains were determined, and the deduced amino acid sequences were 89% identical to that of the original CytK. The authors have designated this new *cytK* variant *cytK-2*, and refer to the original *cytK* as *cytK-1*. The CytK-2 proteins from these three strains were isolated, and their identity was verified by N-terminal sequencing. BLAST analysis using the *cytK-2* gene sequences revealed very high homology with two *cytK-2* sequences in the genomes of *B. cereus* strains ATCC 14579 and ATCC 10987. The differences between CytK-1 and the CytK-2 proteins were clustered to certain regions of the proteins. The isolated CytK-2 proteins were haemolytic and toxic towards human intestinal Caco-2 cells and Vero cells, although their toxicity was about 20% of that of CytK-1. Both native and recombinant CytK-2 proteins from *B. cereus* 1230-88 were able to form pores in planar lipid bilayers, but the majority of the channels observed were of lower conductance than those created by CytK-1. It is likely that CytK-2 toxins contribute to the enterotoxicity of several strains of *B. cereus*, although not all of the CytK-2 toxins may be as harmful as the CytK-1 originally isolated.

Report KMA 1.3/06 Guinebretière, M-H. *et al.* (2002). Enterotoxigenic Profiles of Food-Poisoning and Food-Borne *Bacillus cereus* Strains. Journal of Clinical Microbiology, Vol. 40, No. 8, pp. 3053-3056.

GLP No

Summary

The enterotoxigenic profiles of 51 *B. cereus* food-related strains were compared to those of 37 *B. cereus* food-poisoning strains. *cytK* and association of *hbl-nhe-cytK* enterotoxin genes were more frequent among diarrheal strains (73 and 63%) than among food-borne strains (37 and 33%). Unlike diarrheal strains, food-borne strains showed frequent *nhe* and *hbl* gene polymorphisms and were often low toxin producers.

Report KMA 1.3/04 Ehling-Schulz, M. *et al.* (2006). Cereulide synthetase gene cluster from emetic *Bacillus cereus*: Structure and location on a mega virulence plasmid related to *Bacillus anthracis* toxin plasmid pXO1. BMC Microbiology, Vol. 6, No. 20.

GLP No

Summary

Cereulide, a depsipeptide structurally related to valinomycin, is responsible for the emetic type of gastrointestinal disease caused by *Bacillus cereus*. Recently, it has been shown that this toxin is produced by a nonribosomal peptide synthetase (NRPS), but its exact genetic organisation and biochemical synthesis is unknown. The complete sequence of the cereulide synthetase (*ces*) gene cluster, which encodes the enzymatic machinery required for the biosynthesis of cereulide, was dissected. The 24 kb *ces* gene cluster comprises 7 CDSs and includes, besides the typical NRPS genes like a phosphopantetheinyl transferase and two CDSs encoding enzyme modules for the activation and incorporation of monomers in the growing peptide chain, a CDS encoding a putative hydrolase in the upstream region and an ABC transporter in the downstream part. The enzyme modules responsible for incorporation of the hydroxyl acids showed an unusual structure while the modules responsible for the activation of the amino acids Ala and Val showed the typical domain organisation of NRPS. The *ces* gene locus is flanked by genetic regions with high homology to virulence plasmids of *B. cereus*, *Bacillus thuringiensis* and *Bacillus anthracis*. PFGE and Southern hybridisation showed that the *ces* genes are restricted to emetic *B. cereus* and indeed located on a 208 kb megaplasmid, which has high similarities to pXO1-like plasmids. The *ces* gene cluster that is located on a pXO1-like virulence plasmid represents, beside the insecticidal and the anthrax toxins, a third type of *B. cereus* group toxins encoded on megaplasmids. The *ces* genes are restricted to emetic toxin producers, but pXO1-like plasmids are also present in emetic-like strains. These data might indicate the presence of an ancient plasmid in *B. cereus* which has acquired different virulence genes over time. Due to the unusual structure of the hydroxyl acid incorporating enzyme modules of Ces, substantial biochemical efforts will be required to dissect the complete biochemical pathway of cereulide synthesis.

Report KMA 1.3/03 Böhm, M-E. et al. (2016). Comparative Bioinformatics and Experimental Analysis of the Intergenic Regulatory Regions of *Bacillus cereus hbl* and *nhe* Enterotoxin Operons and the Impact of CodY on Virulence Heterogeneity. Frontiers in Microbiology, Vol. 7, Art. 768.

GLP No

Summary

Bacillus cereus is a food contaminant with greatly varying enteropathogenic potential. Almost all known strains harbour the genes for at least one of the three enterotoxins Nhe, Hbl, and

CytK. While some strains show no cytotoxicity, others have caused outbreaks, in rare cases even with lethal outcome. The reason for these differences in cytotoxicity is unknown. To gain insight into the origin of enterotoxin expression heterogeneity in different strains, the architecture and role of 50 intergenic regions (50 IGRs) upstream of the *nhe* and *hbl* operons was investigated. *In silico* comparison of 142 strains of all seven phylogenetic groups of *B. cereus sensu lato* proved the presence of long 50 IGRs upstream of the *nheABC* and *hblCDAB* operons, which harbour recognition sites for several transcriptional regulators, including the virulence regulator PlcR, redox regulators ResD and Fnr, the nutrient-sensitive regulator CodY as well as the master regulator for biofilm formation SinR. By determining transcription start sites, unusually long 50 untranslated regions (50 UTRs) upstream of the *nhe* and *hbl* start codons were identified, which are not present upstream of *cytK-1* and *cytK-2*. Promoter fusions lacking various parts of the *nhe* and *hbl* 50 UTR in *B. cereus* INRA C3 showed that the entire 331 bp 50 UTR of *nhe* is necessary for full promoter activity, while the presence of the complete 606 bp *hbl* 50 UTR lowers promoter activity. Repression was caused by a 268 bp sequence directly upstream of the *hbl* transcription start. Luciferase activity of reporter strains containing *nhe* and *hbl* 50 IGR *lux* fusions provided evidence that toxin gene transcription is upregulated by the depletion of free amino acids. Electrophoretic mobility shift assays showed that the branched-chain amino acid sensing regulator CodY binds to both *nhe* and *hbl* 50 UTR downstream of the promoter, potentially acting as a nutrient-responsive roadblock repressor of toxin gene transcription. PlcR binding sites are highly conserved among all *B. cereus sensu lato* strains, indicating that this regulator does not significantly contribute to the heterogeneity in virulence potentials. The CodY recognition sites are far less conserved, perhaps conferring varying strengths of CodY binding, which might modulate toxin synthesis in a strain-specific manner.

Report:	**KMA 6.1/05**; Harriam, U. (2015).
Title:	Enterotoxigenic Bacillus cereus and Bacillus thuringiensis Spores in U.S. retail Spices. University of Massachusetts - Amherst.
Document No:	-
Guidelines:	None
GLP	No

Summary

The main objective was to characterise *B. cereus* spores from U.S. retail spices. 247 retail spice samples were analysed. Levels of aerobic spores and *B. cereus* spores were determined using both plant court and most probable number (MPN) techniques. *B. cereus* spores were further analysed for their enterotoxigenic ability, growth characteristics and physical spore characteristics.

Samples contained a wide range of aerobic-mesophilic, bacterial spore-counts that ranged from < 200 to 8.3 x 10^7 CFU/g. 19.1% of samples contain levels above 10^5 CFU/g. Paprika, allspice, peppercorns and mixed spices were some of the spices that were found to possess high levels of aerobic spores (> 10^7 CFU/g).

Using a novel chromogenic agar, *B. cereus* spores were isolated from 77 samples (31%) and *B. thuringiensis* spores were isolated from 11 (4%) samples. Levels of *B. cereus* ranged from <3 MPN/g to 1600 MPN/g. *B. thuringiensis* ranged from 3.6 to 240 MPN/g with only one

sample in the 100-1600 range - the rest were evenly distributed between ranges 1 to 10 and 10 to 100.

Eighty four of the 88 spices tested possessed either one of the enterotoxin genes. None of the isolates tested positive for the emetic toxin (*ces*) gene.

B.6.1.1.1 Medical data

No adverse reactions as a result of contact with *Bacillus thuringiensis* ssp. *aizawai* have been reported in the general population. No cases of adverse effects have been documented in people exposed to *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857). No adverse reactions as a result of contact with *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857) have been reported or documented during development, manufacture, preparation or field application.

B.6.1.1.2 Medical surveillance on manufacturing plant personnel

No adverse reactions as a result of contact with *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857) have been reported or documented during development, manufacture, formulation or field application. A recent report dated August 2016 confirms the absence of adverse reactions observed in or reported by fermentation operators and fermentation pilot plant operators resulting from exposure to XenTari® *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857) at the ■ manufacturing facility (Glynn, 2016). Parameters routinely assessed in operators include spirometry, haematology and clinical chemistry.

Report:	MMA 5.1.2/01, Glynn S (2016)
Title:	XenTari® - Bta
Document No:	NA
Guidelines:	None
GLP:	No

Conclusion: This internal document concludes that no adverse reactions in individuals resulting from contact with XenTari® during its development, manufacture or preparation have been reported or documented. There have been no medical surveillance abnormalities or reports to the Occupational Health Service at the manufacturing or formulation plants relating to XenTari®.

B.6.1.1.3 Sensitisation/allergenicity observations, if appropriate

In common with all microbial active substances, *Bacillus thuringiensis* subsp. *aizawai* (strain ABTS 1857) is considered to be a potential skin and respiratory sensitiser.

Bernstein *et al*. (1999)[15] have previously reported the presence of specific IgE and IgG antibodies to *B. thuringiensis* in exposed farmworkers; however these findings were not associated with any symptoms of skin or respiratory sensitisation. Similarly Doekes *et al*. (2004)[16] report detectable IgE to *B. thuringiensis* in a cohort of exposed Danish greenhouse workers (BIOGART study). No sensitisation reactions in the general population exposed to *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857) have been documented; similarly no sensitisation reactions have been reported or documented during the development, manufacture, preparation or field application of the active substance or products containing the active substance. An additional study (Baelum *et al*., 2012) followed greenhouse workers over three years of exposure to *B. thuringiensis, T. harzianum,* and *V. lecanii.* Exposure to products containing strains of *Bacillus thuringiensis* in greenhouses culturing ornamental gave rise to elevated IgE antibodies but there was no systematic relation to symptoms, in lung function, or in bronchial hyper-responsiveness. The potential for sensitisation is therefore concluded to be minimal.

Report:	MMA 5.1.3/01, Baelum J, Larsen P, Doekes G & Sisgaard T (2012).
Title:	Health effects of selected microbiological control agents. A 3-year follow-up study
Reference:	Annals of Agricultural and Environmental Medicine 19(4). 631-636.
Guidelines:	None
GLP:	No

Abstract: The study assessed the health effects in 579 greenhouse workers of exposure to three types of commonly used biopesticides. Subjects were followed for three years with annual medical examination. Biopesticide exposure was estimated. Serum IgE levels were measured using enzyme immunoassay. The results of this study indicate the presence of specific IgE in greenhouse workers exposed to products containing *B. thuringiensis*, but do not show any effects on the incidence or prevalence of respiratory symptoms, lung function or bronchial responsiveness.

Materials: Products containing *Bacillus thuringiensis, Verticillium lecanii* and *Trichoderma harzenianum.*

Methods: A total of 579 individuals (age 17-67 years) involved in culturing ornamental flowers were included in the study. They were followed for three years with annual examinations including interview about exposure and symptoms, lung function, including bronchial (histamine) challenge test and blood samples. Direct and indirect exposure for each person and year was estimated by information from respondents and employers. IgE in serum against the 7 products of MCBA (including the Bti products Bactimos and Vectobac and Btk product Dipel) was analysed using enzyme immunoassay.

Findings: 65% of the individuals in this study were exposed to products containing *B. thuringiensis*. IgE against *B. thuringiensis* was seen in 53% of the samples, with prevalence rate ratios among those exposed increasing from 1.20 to 1.43 over the 3-year period of the study. There was, however, no association between exposure to any of the biopesticides investigated and the prevalence nor incidence of respiratory symptoms. There was no effect on lung function or bronchial responsiveness.

[15]Bernstein L *et al*., (1999); Immune Responses in Farm Workers after Exposure to Bacillus thuringiensis Pesticides; Environmental Health Perspectives 107(7):575-582.
[16]Doekes G *et al*., (2004). IgE sensitization to bacterial and fungal biopesticides in a cohort of Danish greenhouse workers: the BIOGART study. American Journal of Industrial Medicine 46(4):404-407.

Conclusion: The results of this study indicate the presence of specific IgE in greenhouse workers exposed to products containing *B. thuringiensis*, but do not show any effects on the incidence or prevalence of respiratory symptoms, lung function or bronchial responsiveness.

In earlier work, Pearce *et al*. (2002)[17] investigated whether aerial spraying of Btk was associated with an increase in the symptoms or change in the Peak Expiratory Flow Rate of children with asthma. A pre/post matched pairs cohort design was used, in which children living in the spray zone were matched with children outside the spray zone. Peak Expiratory Flow Rates, asthma symptoms and non-asthma symptoms were recorded in diaries. There were no differences in asthma symptom scores between subjects and controls, neither before nor after the spray; nor were there significant changes in Peak Expiratory Flow Rates for subjects after the spray period. The conclusion was that there was no evidence of adverse effects from the use of the biological pesticide.

The adult gastrointestinal tract is colonised primarily by anaerobic bacteria that broadly belong to four phyla: Firmicutes, Bacteroidetes, Proteobacteria and Actinobacteria. Short-chain fatty acids (SCFAs) such as acetate, butyrate and propionate are major fermentation products of normal gut bacteria, and are the main energy source for colonic epithelial cells. SCFAs are also shown to have a number of beneficial effects, including playing a role in maintaining intestinal homeostasis (Sun & Chang, 2014)[18]. Some aspects of host immune function are also regulated by metabolites produced by the microbiome. Butyrate, one SCFA produced by members of the microbiota, has been shown to facilitate the development of localised immunity (Leslie & Young, 2015)[19].

The development of gut dysbiosis (microbial imbalance) can result in disturbed intestinal homeostasis and may activate host immune and inflammatory responses. The effects of Btk (Strain ABTS-351) have therefore been investigated on the composition of the normal gastrointestinal microflora (reference?), on the production of SCFA metabolites (reference?) and on a model of the immunosuppressed gut (van der Wiele (2015). In the study performed by van der Wiele (2015) is found that Bta did not subvert the expression cascade required for an effective immunological stress response in the simulated colon of an immunocompromised individual.

Studies specifically carried out to study the effects of Btk strain ABTS-351 on 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 (ProDigest (2018). In this recently submitted study (2018) is found that spores of the *Bta* strain ABTS-1857 (XenTari) behave similar to the probiotic control *B. clausii* while behaving very differently from *B. cereus* in terms of germination along the human GIT. While *B. cereus* spores germinated strongly upon exposure to human Caco-2 cells, a markedly lower or even a complete absence of germination was observed for the *Bta* strain ABTS-1857 (XenTari) and *B. claussii*. This especially followed from the experiment involving direct exposure to human cells, where adhesion of Bta strain ABTS-1857 (XenTari) to human cells was shown

[17]Pearce M, Habbick B, Williams J, Eastman M & Newman M (2002). The effects of aerial spraying with *Bacillus thuringiensis Kurstaki* on children with asthma. Canadian Journal of Public Health 93(1):21-25.
[18]Sun J & Chang EB (2014). Exploring gut microbes in human health and disease: Pushing the envelope. Genes & Disease 1:132-139.
[19]Leslie JL & Young VB (2015). The rest of the story: the microbiome and gastrointestinal infections. Current Opinion in Microbiology 23:121-125.

not to induce germination of Bta strain ABTS-1857 (XenTari).

Report:	MMA 5.1/04; van der Wiele *et al*., (2015).
Title:	Evaluation of the germination behavior of DiPel and XenTari during passage through the GIT and their impact on the gut microbiota. *In vitro* study of immune effects mediated by the fermentation-derived metabolites using Caco-2 / THP1 co-cultures
Document No.:	ProDigest, Belgium; Final Report – November 2015
Guidelines:	None
GLP:	No

Abstract: This combined study investigated bacteria-host interactions and interaction with the immune system in SHIME (Simulator of Human Gastro Intestinal Microbial Ecosystem) derived extracts, using a layered co-culture model imitating intestinal epithelial cells (represented by Caco-2 cells) and associated mucosal layer (represented by THP-1 cells).

Material: Extracts from the SHIME model following the administration of antibiotic and XenTari® (*Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857).

Methods: To investigate the activity of Bta strain ABTS 1857 on immune responses in the colon under worst-case conditions, the SHIME system was treated with an antibiotic to simulate the gastric conditions of an immunocompromised individual. Samples were derived from extracts taken at three time points: (1) at the end of a control period once gastrointestinal bacteria achieved relative homeostasis (control); (2) at the end of a 1 week period in which product was fed daily at 10^6 CFU and clindamycin was fed three times daily for five days (Spores+AB); and (3) at the end of a one week period in which only Bt product was fed into the system at 10^6 CFU per day (Spores). SHIME extracts were diluted, filtered to remove spores, and incubated in the co-cultured cell model to measure the onset of a signalling cascade of genes involved in countering inflammation and wound healing. A breakdown in this anti-inflammatory response would result in chronic inflammation.

For the *in vitro* assessment, Caco-2 cells were grown on semi-permeable inserts until maturation and the formation of a functional polarised monolayer. The insert-supported cells were subsequently co-cultured with activation THP-1 macrophages, and lipopolysaccharide (LPS) added to the basolateral side to induce the release of inflammatory mediators and reduce barrier integrity. To this model was added diluted SHIME suspension, from which the bacterial cells had been removed by filtration.

TEER (transendothelial electrical resistance) was measured at 24 hours after to treatment, as a

marker of barrier integrity. Levels of inflammatory mediators (IL-1β, IL-6, IL-10, TNF-α, CXCL10 and MCP-1) were measured using commercial assay. NF-κB activity of the THP-1 macrophages was measured using a reported cell line assay.

Findings: Bta increased TEER indicating reduced permeability and greater stability of Caco-2 cells. Levels of IL-8 were also reduced. Bta treatment additionally reduced the pro-inflammatory protein TNF-alpha. Bta also slightly reduced the expression of the cytokine CXCL10, which attracts immune cells to the site of infection, though this change in expression was not reflective of a common trend. Overall, Bta did not subvert the expression cascade required for an effective immunological stress response in the simulated colon of an immunocompromised individual.

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Report:	MMA 5.1/05; ProDigest (2018).
Title:	Evaluation of the effect of *Bacillus thuringiensis* XenTari on Caco-2 cells (follow up Deal 312)
Document No.:	ProDigest BVBA, Belgium; Ref: 2015099/D615/BTvsBC, May 4th, 2018
Guidelines:	None
GLP:	No

Abstract: This study was carried out to evaluate the potential impact of the *Bacillus thuringiensis* subspecies aizawai, strain ABTS-1857 (Bta) under the name strain XenTari on the modulation of the gut wall, as a follow-up of the experiments previously conducted with this Bta strain ABTS-1857. The work was divided in four phases:
Phase 1. Preparation of spore stocks of *B. cereus* and *B. clausii*
Phase 2. Germination capacity of XenTari spores in HBSS minimal medium
Phase 3. Germination capacity of XenTari spores in HBSS minimal medium collected after incubation with Caco-2 cells (~ germination upon indirect exposure to human cells)
Phase 4. Germination capacity of XenTari spores in HBSS minimal medium in the presence of Caco-2 cells (adhesion) (~ germination upon direct exposure to human cells)
In each test, the results of Bta strain ABTS-1857 (XenTari) were compared to a known pathogenic (*Bacillus cereus* (ATCC 14579)) and probiotic (*Bacillus clausii*) strains.

Overall, these data support that the spores of the Bta strain ABTS-1857 (XenTari) behave similar to the probiotic control *B. clausii* while behaving very differently from *B. cereus* in terms of germination along the human GIT. While *B. cereus* spores germinated strongly upon exposure to human Caco-2 cells, a markedly lower or even a complete absence of germination was observed for the Bta strain ABTS-1857 (XenTari) and B. claussii. This especially followed from the experiment involving direct exposure to human cells, where adhesion of Bta strain ABTS-1857 (XenTari) spores to human cells was shown not to induce germination of XenTari.

Material: XenTari® (*Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857).

Methods: A number of experiments were performed in order to determine the potential germination of the Bta strain ABTS-1857 (XenTari) versus the pathogenic and probiotic control upon exposure to solely germinants released by the Caco-2 cells. For this purpose, a project consisting of four phases was designed:

Phase 1. Preparations of spore stocks of *B. cereus* and *B. clausii.*

The strains were grown overnight in pre-sporulation medium (containing tryptone and yeast extract) at 30°C, after which they were plated on sporulation agar plates (containing nutrient broth, yeast extract and MnCl2). After 10 days of incubation at 30 °C, spores were harvested, washed and resuspended in 96% cold ethanol. After a next washing step, they were finally suspended in saline supplemented with 0.1% peptone.

Phase 2. Germination capacity of Bta strain ABTS-1857 (XenTari) spores in minimal medium
This test was intended to assess the germination capacity of XenTari in HBSS (Hank's Balanced Salt Solution) minimal medium. 10^6 CFU spores/mL from all 3 strains were in HBSS without any supplements for either 2h or 4h at 37°C. Serial dilutions were made and plated as such (= TVC = sum of spores and vegetative cells). In addition, dilutions were also plated upon incubation of the dilutions for 20 min at 80°C to remove any vegetative cells that were present, thus allowing to specifically determine the amount of spores being present.

Phase 3: Germination capacity of Bta strain ABTS-1857 (XenTari) spores in HBSS minimal medium collected upon incubation with Caco-2 cells
Caco-2 cells were incubated in HBSS for either 4h or 16h, during which the Caco-2 cells released potential host-derived germinants in the medium, after which supernatants were collected. A WST1 assay was performed to determine cell viability of the Caco-2 cells upon incubation with HBSS.

Phase 4: Germination capacity of Bta strain ABTS-1857 (XenTari) spores in HBSS minimal medium in the presence of Caco-2 cells (adhesion)
In this final phase, the germination potential of the Bta strain ABTS-1857 (XenTari) was assessed upon direct exposure to human enterocytes. Caco-2 cells were cultured as described in Phase 3. Subsequently, 10^6 CFU spores/mL of all three *Bacillus* strains in fresh HBSS minimal medium were added to the Caco-2 cells and incubated for 4h at 37 °C. Serial dilutions of the medium were made and plated as such (= TVC) or upon heat treatment to specifically quantify spores, as elaborated above. Further, upon removal of non-adherent spores, Caco-2 cells were lysed with saponin, to determine the number of adhered spores and vegetative cells.

Findings: After producing the required spore stocks (phase 1), the germination potential of three strains (Bta strain ABTS-1857 (XenTari), *B. cereus* and *B. clausii*) was tested in minimal medium (HBSS) (phase 2). This revealed that the probiotic *B. clausii* and the Bta strain ABTS-1857 (XenTari) did not germinate in this minimal medium. Therefore, the HBSS medium was confirmed to be a proper medium for investigating the effect of specific germinants that are added to this minimal medium, *in casu* host-derived factors. The pathogenic *B. cereus* was able to germinate in minimal medium (HBSS), displaying the strong inherent germination capacity of this pathogenic strain.

During the third phase of the project, germination of the *Bacillus* spores upon indirect exposure to human cells was investigated. For this purpose, supernatants were collected upon incubating HBSS medium during 4h and 16h with Caco-2 cells. While the 4h sample contained cell factors released by a perfectly intact epithelial cell layer, the 16h sample contained factors released from less viable epithelial cells thus potentially containing extra germinants that are released upon cell lysis. To get maximal insights in the research question, both supernatants (4h and 16h) were tested. Overall, it was found that the host-derived factors resulted in strong germination of the pathogenic *B. cereus* and to a lower extent also

of Bta strain ABTS-1857 (XenTari). In contrast, when focusing on the 2h incubation time, no germination was observed for the probiotic *B. claussii*.

During the fourth phase of the project, the most representative research question was addressed for the *in vivo* situation, i.e. germination of the *Bacillus* spores upon direct exposure to human cells. For this purpose, spores were incubated in HBSS medium in the presence of Caco-2 cells for 4h. Whereas direct incubation with the cells resulted in strong germination of the pathogenic *B. cereus* in the medium, no germination was observed for the probiotic *B. claussi* and the Bta strain ABTS-1857 (XenTari). Also, adhesion to the cells did not induce germination of Bta strain ABTS-1857 (XenTari). Finally, addition of Bta strain ABTS-1857 (XenTari) spores to the cells, in contrast to *B. cereus*, did not induce cell death during the 4h incubation period.

Overall, these results indicate that upon direct exposure to human cells, the BT strain Bta strain ABTS-1857 (XenTari) behaved as the probiotic control *B. clausii* and did not germinate in the medium. Also, adhesion to the cells did not induce germination of Bta strain ABTS-1857 (XenTari).

Conclusion: Overall, these data support that the spores of the Bta strain ABTS-1857 (XenTari) behave similar to the probiotic control *B. clausii* while behaving very differently from *B. cereus* in terms of germination along the human GIT. While *B. cereus* spores germinated strongly upon exposure to human Caco-2 cells, a markedly lower or even a complete absence of germination was observed for the Bta strain ABTS-1857 (XenTari) and *B. claussii*. This especially followed from the experiment involving direct exposure to human cells, where adhesion of Bta strain ABTS-1857 (XenTari) to human cells was shown not to induce germination of Bta strain ABTS-1857 (XenTari).

B.6.1.1.4 Direct observation, e.g. clinical cases

Food poisoning

There are no reports of clinical cases due to *Bacillus thuringiensis* ssp. *aizawai* (Strain ABTS 1857). As noted previously, cases of food poisoning due to *B. cereus* and *B. thuringiensis* are not routinely distinguished. It is possible, therefore that cases due to *B. thuringiensis* are under-reported. The lack of production of the emetic toxin by *B. thuringiensis* including ssp. *aizawai* (Strain ABTS 1857) means that food poisoning cases involving emetic syndrome (nausea and/or vomiting) are unlikely to be caused by *B. thuringiensis*. Food poisoning cases involving diarrhoeal syndrome (gastrointestinal pain and diarrhoea) could theoretically be caused by strains of *B. thuringiensis*, although there is no strong evidence for this. The EFSA BioHaz Document points to two cases, both of which are considered to have flaws, such as norovirus also being present. While it is impossible to prove a negative, it is important to note that all the preceding information provides a strong weight of evidence for Bta (Strain ABTS 1857) not being capable of causing a diarrheal event.

Bacillus thuringiensis ssp. *aizawai* (Strain ABTS 1857) can produce low levels of diarrhoeal enterotoxin under very specific culture conditions; however this is unlikely to occur following the proposed use. Furthermore it is notable that long-incubation (diarrhoeal) *B. cereus* food poisoning is associated with the consumption of contaminated meat, milk or high starch foods such as rice and pasta, and is less commonly associated with the consumption of vegetable crops treated with *Bacillus thuringiensis* strains used for agricultural biocontrol. The EFSA BIOHAZ document (EFSA, 2016) reports that

vegetables and vegetable products accounted for <5% of the 413 'strong evidence' food poisoning cases involving presumptive *B. cereus* and reported in EU Member States between 2007-2014. This low incidence contrast with the extensive use of *Bacillus thuringiensis* strains in agricultural biocontrol. Cultivation soil containing the ubiquitously present *B. cereus* is reported to be an important source of vegetable cultivation (Guinebretière & Nguyen-Thé, 2003)[20]; this may therefore be a more likely causative factor in food poisoning incidents lined to the consumption of vegetables.

Jackson *et al*. (1995)[21] found bacterial isolates presumptively identified as *B. cereus* from four persons during the investigation of a gastroenteritis outbreak in a chronic care institution in Canada. The symptoms were nausea, vomiting and watery diarrhoea. Phage typing confirmed all the clinical *Bacillus* isolates to be type 2. The food consumed was not directly analysed. All 10 clinical isolates (from the stool) were subsequently identified as *B. thuringiensis*, as demonstrated by the formation of crystals and observed by staining and microscopy. This suggests, but does not prove, the involvement of *B. thuringiensis* in the gastroenteritis, as *B. cereus* (later identified as *B. thuringiensis*) was the only pathogenic bacteria isolated from the stool of three of the individuals. In the fourth individual, Norovirus was also identified and was found in one individual negative for the presence of *B. cereus* or other pathogens. None of the *B. thuringiensis* isolates examined in this investigations were characterised further than to species level, so their phylogenetic relations within the *B. cereus* group are unknown. The possible involvement of strains used as biopesticides was not investigated. The symptoms described in this paper include nausea and vomiting, which would not indicate *B. thuringiensis* as the causative agent.

McIntyre *et al*. (2008) reanalysed frozen *B. cereus*-like isolates associated with food poisoning incidents in British Columbia, and characterised different *Bacillus* species. *B. thuringiensis* was characterised based on the presence of insecticidal crystal protein (ICP) cry1 or cry2 by microscopy or PCR. Based on this analysis, the authors report that *B. thuringiensis* may have been responsible for 4 of 39 (~10%) food poisoning outbreaks.

The EFSA BIOHAZ document (EFSA, 2016) provides a detailed analysis of a 2012 food poisoning case involving three members of a family of five who had eaten cheese noodles and salad during the evening and suffered symptoms (nausea and diarrhoea) early the following morning (1 a.m.). Symptoms were only reported by individuals who had eaten the salad. Analysis revealed contamination of the noodles with *B. cereus* (6×10^3/g) and contamination of the salad with *B. thuringiensis* (3×10^4/g). Further investigations revealed levels of *B. thuringiensis* in two lettuces from the same retail source of 4×10^4 and 1.5×10^5/g; it was confirmed that the crop had been treated with the product XenTari®, containing *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857). The document also refers to a study in which the presence of the enterotoxin *cytK2*, *nhe* and *hbl* genes, but not the emetic toxin *ces* gene was demonstrated in *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857). While symptoms of food poisoning were reported only by family members who had eaten the *B. thuringiensis*-contaminated salad, the fact that nausea was reported by all those affected indicates that this organism is not the causative factor. All of the affected family members also consumed the *B. cereus*-contaminated noodles; the fact that only three of the five experienced symptoms may reflect differences in individual sensitivity or differences in the quantity consumed. Although the level of *B. cereus* contamination reported (6×10^3/g) is less than that typically associated with food poisoning (1×10^5/g), the EFSA BIOHAZ document also notes that some cases have reported symptoms at lower levels of contamina-

[20]Guinebretiere MH & Nguyen-The C (2003). Sources of *Bacillus cereus* contamination in a pasteurized zucchini purée processing line, differentiated by two PCR-based methods. FEMS Microbiology Ecology 43(2):207-215.

[21]Jackson SG et al., (1995). *Bacillus cereus* and *Bacillus thuringiensis* isolated in a gastroenteritis outbreak investigation. Letters in Applied Microbiology 21(2):103-105.

tion (1 x10^3/g). This is likely to reflect the variability in the level of toxin production among *B. cereus* strains. Furthermore, 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) (reference?), it would seem very unlikely that this is the causative agent. The EFSA BIOHAZ document also notes that the symptoms of nausea in this particular case do not indicate *B. thuringiensis* as the causative factor, a synergistic effect between *B. cereus* and *B. thuringiensis* is suggested. However, based on the low level of exotoxin (??) production, it is unlikely that *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857) made any contribution to the symptoms reported in this case (see also B.6.1.1 with the conclusion 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).

Exposure from biopesticide use

Forrester (2012) reports a lack of serious incidents resulting from exposure to *Bacillus thuringiensis* pesticides.

Report:	MMA 5.1.4/01, McIntyre L, Bernard K, Beniac D, Isaac-Renton JL & Naseby DC (2008).
Title:	Identification of *Bacillus cereus* Group Species Associated with Food Poisoning Outbreaks in British Columbia, Canada.
Reference:	Applied & Environmental Microbiology 74(23): 7451-7453
Guidelines:	None
GLP:	No

Abstract: In this study, the authors reanalysed frozen *B. cereus*-like isolates associated with food poisoning incidents in British Columbia, and characterised different *Bacillus* species. *B. thuringiensis* was characterised based on the presence of insecticidal crystal protein (ICP) Cry1 or Cry2 by microscopy or PCR. Based on this analysis, the authors report that *B. thuringiensis* was responsible for 4 of 39 (~10%) food poisoning outbreaks.

Material: Frozen *B. cereus*-like isolates associated with food poisoning incidents.

Methods: Frozen isolates were cultured on blood agar and subject to phenotypic characterisation. Pathogenicity genes for the emetic toxin cereulide (non-ribosomal peptide synthetase (NRPS)) and ICP (cry1 or cry2) were detected using multiplex PCR assays Strains positive for NRPS were designated as *B. cereus*; *B. thuringiensis* was characterised based on the presence of insecticidal crystal protein (ICP) cry1 or cry2 by microscopy or PCR.

Findings: Based on the phenotypic characterisation, the authors report that *B. thuringiensis* was responsible for 4 of 39 food poisoning outbreaks previously attributed to *B. cereus*.

Conclusion: Based on this analysis, it is possible that a small proportion (~10%) of food poisoning outbreaks previously attributed to *B. cereus* may have been caused by *B. thuringiensis*; however distinguishing between the species requires non-standard investigations. Nevertheless, it is known that spores can remain in samples, well after other microorganisms have died. Furthermore, the identification of a ubiquitous microorganism in food samples does not demonstrate cause and effect. It is notable that there are nu-

merous publications which identify *B. cereus* and *B. thuringiensis* in stool samples of healthy individuals.[22,23,24]

Report:	MMA 5.1.4/02, Forrester MB (2012)
Title:	*Bacillus thurengiensis* pesticide exposures reported to Texas poison centers
Reference:	Toxicological & Environmental Chemistry 94(4): 799-804
Guidelines:	Not applicable
GLP:	No

Abstract: The author describes the *Bacillus thuringiensis* pesticide exposures reported to Texas poison centres between 2000-2010; the distribution of cases reported was determined for various demographic and clinical factors.

Methods: All cases of effects resulting from exposure to *Bacillus thuringiensis* pesticides reported to the Texas Poison Center Network by telephone from 2000-2010 were analysed for cause and medical outcome, among other factors.

Findings: A total of 115 *Bacillus thuringiensis* pesticide exposures were identified. The annual number of exposures ranged from 3-18, with no trend apparent. The majority of the exposures were reported between April and July. Of the patients involved, 56% were ≥20 years old and 55% were male. The exposure routes involved were ingestion (57%), dermal contact (30%) and inhalation (11%). Exposures were classed as unintentional (97%), intentional (3%) and an adverse reaction (1%). Distribution by medical outcome was no effect (22%), minor effect (9%), not followed and judged to be non-toxic (9%), not followed and minimal effects expected (54%), unable to follow and judged to be potentially toxic (1%) and effects unrelated to exposure (6%). There were no cases with moderate or major clinical effects. The most frequently reported effects were diarrhoea, nausea, abdominal pain or vomiting; skin, eye or throat irritation. A high proportion of the incidents reported related to the use of 'Mosquito Dunks'; floating rungs used for mosquito control.

Conclusion: The authors conclude that the majority of *Bacillus thuringiensis* biopesticide exposures reported to Texas poison centres did not result in serious outcomes.

[22]Jensen GB *et al*. (2002). *Bacillus thuringiensis* in Fecal Samples from Greenhouse Workers after Exposure to *B. thuringiensis*-Based Pesticides. Applied & Environmental Microbiology 68(10):4900-4905.

[23]Gosh AC (1978). Prevalence of *Bacillus cereus* in the faeces of healthy adults. Journal of Hygiene 80(2):233-236.

[24]Yea C-L *et al*. (1994). Isolation of *Bacillus cereus* in the feces of healthy adults in Taipei City. Chinese Journal of Microbiology & Immunology 27:148-151.

B.6.1.2 Basic studies

B.6.1.2.1 Sensitisation

The following study of skin sensitisation, performed using XenTari® Technical Powder (ABG-6305) (*Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857)), was assessed in the EU Review and was presented in the DAR (final addendum Feb 2013).

Copy from final addendum:

A study, namely [redacted] (1997a) XenTari Technical Powder (ABG-6305): Dermal sensitization study in Guinea pigs. [redacted]. Unpublished report No.: 3088-96 , is submitted, but it is related to the technical product and not to the active ingredient. Finally according to EU Commission Directive 36 / 2001 /EC the ~~product~~ active should be classified as sensitizer.

A more detailed evaluation of the same study is presented below:

Report:	IIM 5.3.1/01[redacted] (1997a)
Title:	XenTari Technical Powder (ABG-6305): Dermal sensitization study in Guinea pigs
Document No:	3088-96
Guidelines:	US EPA Guideline 81-6 (comparable to OECD 406)
GLP:	Yes

Abstract: The potential of XenTari® Technical Powder ABG-6305 (6.15 x10^{10} CFU/g) was investigated in the guinea pig, according to the Buehler method. Topical induction was performed on a group of five animals/sex using occlusive 6-hour applications of 400 mg of the test material (moistened with distilled water). A total of three induction applications were made to the same shorn dorsal site at weekly intervals. Challenge applications were made two weeks following the final induction application using occlusive 6-hour applications of 400 mg of the test material (moistened with distilled water). The test material was applied for 6 hours under occlusive conditions to a previously untreated site on the shorn dorsal skin of the test animals and a control group (5/sex). Dermal reactions were assessed at 24 and 48 hours following patch removal. No dermal reactions were observed in any of the control or test animals following the challenge application.

Conclusion: No evidence of skin sensitisation was seen under the conditions of this Buehler study.

No new skin sensitisation data are available and no further data are required for this endpoint. The available skin sensitisation study gives a negative response; however it is recognised that the study design may not be optimal for the assessment of microbial substances. As a general principle, it is considered that microbial active substances are potential skin and respiratory sensitisers; therefore all products containing microbial active substances must carry a standard label phrase "contains B. thuringiensis subsp. aizawai. Micro-organisms may have the potential to provoke sensitising reactions."

Studies of respiratory sensitisation are not available; this is not a standard data requirement and there is no agreed standard method for the assessment of respiratory sensitisation.

The available sensitisation data meet the requirements specified in Part B of Regulation (EU) No 283/2013 and no further data are required.

B.6.1.2.2 Acute toxicity, pathogenicity and infectiveness

Acute oral toxicity, pathogenicity and infectiveness

The following acute oral toxicity and pathogenicity study, performed using *Bacillus thuringiensis* ABG-6305 ((*Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857)), was assessed in the EU Review and was presented in the DAR.

Information from the DAR, in a slightly different format

Report:	IIM 5.3.2/01[redacted] (1995)
Title:	Acute oral toxicity/pathogenicity study of *Bacillus thuringiensis* ABG-6305 in rats
Document No:	M94AG90.222.008
Guidelines:	US EPA Guideline Subdivision M 152A-10
GLP:	Yes

Study design

Test material: *Bacillus thuringiensis* ABG-6305 (Lot No.: 84224V9, total spore count 1.83 x 10^{10} CFU/g).

Twenty-two male and twenty-two female Sprague Dawley rats (bodyweights not reported) were individually housed in polycarbonate cages. The animals were allocated to one of two test groups; the treated group contained 18 male and 18 female rats and a control group (room control and shelf control) containing 4 male and 4 female rats.
The treated group was dosed with a single oral dose (10 ml) of *Bt* ABG-6305 at an average concentration of 7.9 x 10^{7} CFU/ml via gavage. All control animals were left untreated.

Clinical observations were recorded 1 and 4 hours after dosing and then once daily thereafter. Bodyweights were recorded prior to dosing and again on days 4, 8, 15, 22 and 29 post dosing.

To evaluate infectivity in the treated group, groups of 6 rats (3 male, 3 female) were sacrificed at regular intervals for testing. Rats were first sacrificed one hour after dosing and further groups of rats were sacrificed on days 3, 7, 14, 21 and 28. Control rats (1 per sex) were sacrificed 3 and 7 days after dosing and the shelf control rats were sacrificed at study termination.
Observations of gross lesions were made for all animals sacrificed for infectivity. Blood and tissues (lymph nodes, kidney, spleen, liver, lungs, kidney and brain) were collected and prepared for plating. Faecal and caecal samples were also plated. Plates were produced in duplicate.

To establish a starting point for clearance, six animals (3 per sex) from the treated group were sacrificed within an hour of dosing and the stomach contents plated in duplicate for colony counts. A further six animals (3 per sex) were placed in metabolism cages and the faeces collected overnight on test days 2 and 3. Additional faecal samples were collected on days 4, 8, 15, 22 and 29. Faecal samples were homogenised and plated for colony counting.

Findings

Bodyweights: No statistically significant differences ($p \leq 0.05$) in bodyweights or bodyweight change were observed in male or female rats throughout the study, when compared to the controls.

Clinical signs: There were no mortalities throughout the duration of the study. Alopecia between the ears was observed in one treated male animal from day 18 until scheduled sacrifice on day 22.

Necropsy: No abnormal findings were noted in any animals at necropsy.

Infectivity: The test microbe was recovered in the stomach of the six treated animals sacrificed on day 1 of the study. Faeces collected from six treated animals on day 2 of the study showed the presence of the test microbe at CFU/g ranging from 4.0 x 106 to 7.6 x 106, no test microbe was found in the faeces from the

control animals. On day 3 of the study the test microbe was found in faeces from two male and three female rats while the controls tested negative.

On day 4, normal flora interfered with the counting of the test microbe in faeces and caecum samples. On day 8, the test microbe was detected in the faeces from two treated animals (1 per sex) and the caecal contents of 4 treated animals (2 per sex). No test microbe was detected in the faeces and caecum of treated animals after day 8. The remainder of the tissues and samples taken from the treated animals showed no test microbe growth. No test microbe was detected in any tissues or samples collected from control animals.

Conclusion: Clearance was demonstrated in the faeces and caecal contents. No signs of infectivity, toxicity or pathogenicity were observed therefore *Bacillus thuringiensis* ABG-6305 was considered to be non-toxic and non-pathogenic in this test system. *Bacillus thuringiensis* ABG-6305 was not infectious in the rat under the conditions of the study.

The following acute oral toxicity study, performed using XenTari® Technical Powder (ABG-6305) ((*Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857)), was assessed in the EU Review and was presented in the DAR

Information from the DAR, in a slightly different format:

Report:	IIM 5.3.2/02[redacted] (1996a)
Title:	XenTari Technical Powder (ABG-6305): Acute oral toxicity study in rats
Document No:	3084-96
Guidelines:	US EPA Guidelines 81-1
GLP:	Yes

Study design: Test material: ABG-6305 Technical powder (Lot No.: 14-455-V9, Total microbial count: 6.15×10^{10} CFU/g).

Five male and five female HSD:SD rats (bodyweight range: 270-287 g for males and 190-204 g for females) were housed individually in cages. Animals were dosed with ABG-6305 Technical powder at a dose equivalent to 5050 mg/kg (approximately 6 x 109 CFU). The rats were administered test material by gavage at dose volume of 16.8 ml/kg in deionised water.

Animals were observed for mortalities and clinical signs of toxicity. Animals were examined 3 times on the day of dosing and then daily thereafter for the duration of the study. Individual bodyweights were recorded prior to dosing and again on days 7 and 14.

At study termination all animals were sacrificed and subjected to gross necropsy examinations.

Findings: There were no mortalities during the study. The main clinical finding was piloerection; decreased activity, crust around the eyes, nose and mouth, respiratory gurgle and staining around the muzzle were also noted in some animals. Very slight to slight diarrhoea was

observed in one female. All animals had made a full recovery by Day 6. There were no treatment related effects on weight gain. No abnormal findings were noted in any animal at necropsy.

Conclusion: Under the conditions of the study, the acute oral LD_{50} of ABG-6305 Technical powder in rats was found to be >5050 mg/kg bw.

The available acute toxicity and pathogenicity data meet the requirements specified in Part B of Regulation (EU) No 283/2013 and no further data are required.

Acute inhalation toxicity, pathogenicity and infectiveness

The following acute inhalation toxicity study, performed using XenTari® Technical powder (ABG-6305) ((*Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857)), was assessed in the EU Review and was presented in the DAR.

Information from the DAR, in a slightly different format:

Report:	IIM 5.3.3/02, [redacted] (1999)
Title:	XenTari Technical Powder (ABG-6305): Acute inhalation toxicity study in rats
Document No:	4432-98
Guidelines:	US EPA Guidelines 81-3
GLP:	Yes

Study design: Test material: XenTari Technical Powder (Lot No.: 39-637-V9, total microbial count: 7.3 x 10^{10} CFU/g).

Five male and five female Sprague Dawley rats (bodyweight range: 245-274g for males 180-199g for females) were housed individually in wire mesh cages. During exposure the test animals were individually placed into polycarbonate exposure tubes which were inserted into a 500 L stainless steel nose only chamber.

During the test the animals were exposed to XenTari Technical Powder (ABG-6305) generated as an aerosol at a concentration of 5.33 mg/L (3.9 x 108 CFU/L) for 4 hours. The test concentration was determined gravimetrically twice an hour during exposure and nominally at the end of the exposure. Particle size was determined twice during the exposure.

The rats were observed frequently during the exposure period and thereafter once daily during the 14 day observation period. Individual bodyweights were recorded prior to exposure and again on days 7 and 14. At study termination animals were sacrificed and subjected to gross necropsy examinations.

Findings: All rats survived the duration of the study. During the 4-hour exposure period, rats displayed decreased activity and piloerection. All rats had made a full recovery by Day 1 of the exposure period. There were no treatment-related effects on body weight gain. Necropsy findings were limited to one male with discoloured lungs.

Conclusion: Under the conditions of the study, the acute inhalation LC_{50} of ABG-6314 in rats was greater than 5.33 mg/L.

The following acute inhalation toxicity and pathogenicity study, performed using *Bacillus thuringiensis* ABG-6305 ((*Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857)), was assessed in the EU Review and was presented in the DAR.

Information from the DAR, in a slightly different format:

Report:	IIM 5.3.4/02, [redacted] (1991a)
Title:	Acute pulmonary toxicity and infectivity/pathogenicity to rats of *Bacillus thuringiensis* ABG-6305
Document No:	901292D/ABT 143-2/AC
Guidelines:	US EPA Guidelines Subdivision M 152A-12
GLP:	Yes

Study design: Test material: *Bacillus thuringiensis* ABG-6305 (Lot No.: 42-211-BD, bacterial count: 2 x 10^{11} CFU/g).

Twenty-three male and twenty-three female Crl:CD (SD) rats (bodyweight range 246-304g) were housed in groups of five of the same sex in wire mesh cages. These rats were allocated to the test material dose group. A group of eight rats (4 male and 4 female) were allocated as the untreated control and a further group of fourteen rats were used as procedural controls.

Test group rats were dosed with *Bacillus thuringiensis* ABG-6305 at 5.33 x 10^8 CFU/ml (10^8 CFU/animal). Dosing was performed under anaesthesia, animals were restrained and a miniature laryngoscope was used to hold open the mouth and a length of translucent tubing was inserted into the trachea. The phases of respiration were identified by the appearance (exhalation) and disappearance (inspiration) of condensation on the walls of the tube. The test suspension was then introduced and injected at the beginning of several inspirations until the entire dose volume had been instilled.

The test animals were observed daily for signs of mortality and clinical signs of toxicity. Individual animal bodyweights were recorded on days 1, 4, 8, 15, 22, 36 and 50.

Three male and three females from the test group animals were sacrificed on days 1 (1 hour after dosing), 4, 8, 15, 22, 36, 50 and subjected to a macroscopic post mortem examination. Samples of blood, brain, lungs, liver, spleen, kidneys, lymph nodes and samples of the caecal contents were removed for microbiological assay.

Findings: All rats survived the duration of the study.

Clinical signs observed included piloerection, waddling, lethargy and decreased respiration immediately post dosing, which could be attributed to the anaesthetic used in the dosing procedure. Only piloerection was observed in rats after Day 12 and all rats made a full recovery by Day 22.

There were no treatment-related effects on body weight gain.

There were no abnormal findings at necropsy. One hour after dosing, substantial numbers of viable organisms were detected in the lungs of the selected rats. By Days 35 and 49, viable organisms could not be detected in the lungs of the sampled rats. No viable organisms were detected in any of the rats 3 days after dosing. Seven days after dosing two rats showed low numbers of viable organisms in the kidneys and one rat showed viable organisms in the spleen. Thereafter, one rat showed low numbers of viable organisms in the spleen 14 days after dosing. No viable organisms were isolated from any of the other tissues and organs of any of the remaining treated rats. Moderate numbers of viable organisms were recovered from all rats until 21 days after dosing; no viable organisms were re-

covered from samples 35 days after dosing. On Day 49 post dosing, low numbers of viable organisms were recovered from five of the ten rats sampled. Ingestion of viable organisms as a result of mucociliary clearance from the lungs was considered to give rise to the recovery of moderate numbers of viable organisms from caecal contents over a sustained period of 21 days. The recovery of low numbers of viable organisms from some rats at 49 days was attributed to individual rat variation and not indicative of intestinal colonisation by *Bacillus thuringiensis* ABG-6305.

Conclusion: Under the conditions of the study, *Bacillus thuringiensis* ABG-6305 showed mild toxicity and no pathogenicity or infectivity following intratracheal dosing. Complete clearance was seen at Day 35 after intratracheal administration.

The available acute inhalation toxicity, pathogenicity and infectiveness data meet the requirements specified in Part B of Regulation (EU) No 283/2013 and no further data are required.

Intraperitoneal/subcutaneous single dose

The following intraperitoneal and subcutaneous injection toxicity study, performed with ABG-6305 Technical Powder ((Bacillus thuringiensis ssp. aizawai (strain ABTS 1857)), was assessed in the EU Review and was presented in the DAR.

Information from the DAR, in a slightly different format:

Report:	IIM 5.3.4/01, [redacted] (1990)
Title:	Intraperitoneal and subcutaneous injection tests with ABG-6305 Technical Powder
Document No:	85K-11/9
Guidelines:	US EPA Guidelines Subdivision M 151A-10
GLP:	Yes

Study design: Test material: ABG-6305 Technical Powder (Lot No.: 42-221-BD, microbial count 2 x 10^{11} CFU/g).

Groups of 5 male and five female mice (bodyweight range 18-22 g) were given an intraperitoneal dose of ABG-6305 Technical Powder at 0.005, 0.05 and 0.5 mg/animal which was equivalent to 1 x 10^6, 1 x 10^7 and 1 x 10^8 CFU/animal respectively.

A second group of animals (5 females, bodyweight range: 21-23g) were administered a 0.25 ml dose containing 1 x 10^7 cfu/animal (actual amount 0.0625 mg/animal) by the subcutaneous route.

Animals were observed 1, 2 and 4 hours post dosing and then twice daily thereafter (weekdays) and once daily (weekends) for at least one week after dosing for signs of toxicity. Seven days after dosing the study was terminated and the animals were sacrificed. There were no necropsy examinations performed.

Findings: There were no mortalities throughout the duration of the study for both the intraperitoneal and subcutaneously treated animals. There were no signs of toxicity noted in any test animals throughout the duration of the study.

Conclusion: No signs of toxicity, pathogenicity or infectivity were seen in mice receiving 25 mg/kg bw (5x10^9 CFU/kg bw) or 3 mg/kg bw (5x10^8 CFU/kg bw) by the intraperitoneal and subcutaneous routes respectively.

The following intravenous injection toxicity and pathogenicity study, performed with ABG-6305 ((*Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857)), was assessed in the EU Review and was presented in the DAR.

Information from the DAR, in a slightly different format:

Report:	IIM 5.3.4/02, [redacted] (1991b)
Title:	Acute intravenous toxicity and infectivity/pathogenicity to rats of ABG-6305
Document No:	901291D/ABT 143-1/AC
Guidelines:	US EPA Guidelines 152A-13
GLP:	Yes

Study design: Test material: *Bacillus thuringiensis* ABG-6305 (Lot No.: 42-221-BD, Potency: 2 x 10^{11} CFU/g).

Twenty-one male and twenty-one female CD rats (bodyweight range: 115 – 133g) were allocated to one of seven test groups each containing three males and three females. All the test animals were dosed with 2.94 x 10^7 cfu/ml at a dose rate of 3.0 ml/kg (1 x 10^7 CFU/mL). A further group of sixteen rats (eight male and eight female) were used as untreated controls. Animals were observed soon after dosing and then at hourly intervals for the remainder of the first day of dosing. On subsequent days animals were observed twice a day. Clinical signs were recorded at each observation point. Bodyweights were recorded on days 1, 4, 8, 22, 29, 36, 43, 50, 57, 64 and 67.

Groups of three male and three female rats were sacrificed 1 hour after dosing, and on days 4, 8, 15, 22, 36 and 67. All sacrificed animals were subjected to macroscopic post mortem examinations. Samples of the brain, lungs, liver, spleen, kidneys, lymph nodes and caecal contents were removed from all the sacrificed rats and subjected to microbiological assay.

Blood, tissue and caecal content samples were prepared for analysis of *Bacillus thuringiensis* ABG-6305 and the numbers of viable spores per gram of tissue was calculated.

Findings: All animals survived the duration of the study. Piloerection was observed in all treated rats within four minutes of dosing and continued throughout the remainder of Day 1. All animals made a full recovery by Day 2. There were no further clinical signs.

There were no treatment-related effects on body weight gain and no abnormal findings at necropsy. Three days after dosing, viable organisms were isolated from all samples, with the largest numbers of viable cells detected in the spleen, liver and lungs with moderate numbers persisting in the mesenteric lymph nodes and kidneys. Lower numbers of viable organisms were isolated from the brain and blood. Fourteen days after dosing no viable organisms were isolated from any blood samples, and a significant decline in the numbers of viable organisms present in brain and kidney samples was observed. Results obtained 21 and 35 days after dosing were similar, showing that viable organisms were not isolated from any brain samples and a gradual decline in the numbers of viable organisms isolated from mesenteric lymph nodes, lungs and liver samples was observed. At study termination, 66 days after dosing, no viable organisms were recovered from the blood, brain, kidneys and livers, with low numbers recovered from the lung. Three rats showed little or no recovery of viable organisms from mesenteric lymph nodes after 66 days, the remaining three rats showed persistence of moderate numbers of viable organisms at levels only marginally lower than those recovered at earlier time points. All spleen samples showed a decline in the numbers of viable organisms isolated. The rate of clearance of viable organisms from the spleen over the study period was calculated to be 6.2% per day, with a half- life of 10.8 days. Low numbers of viable organisms were recovered from rats sacrificed at 7, 14 and 21 days after dosing. With the exception of one rat, no viable organisms were recovered from any samples at later time points.

Conclusion: Under the conditions of the study, mild clinical signs of toxicity (piloerection) but no signs of pathogenicity or infectivity were observed in rats receiving 10^7 CFU. Incomplete clearance from the spleen and lungs was seen after 66 days.

Clearance of the microorganism was not assessed in the intraperitoneal/subcutaneous study, but was assessed in the intravenous study. Although the clearance is incomplete from the spleen, all spleen samples showed a decline in the numbers of viable organisms isolated. The rate of clearance of viable organisms from the spleen over the study period was calculated to be 6.2% per day, with a half- life of 10.8 days.

Together, the studies meet the requirements of Part B of Regulation (EU) No 283/2013 and no further data are required.

B.6.1.2.3 Genotoxicity testing

Standard genotoxicity assays are not considered to be appropriate for testing the genotoxicity of microorganisms. Part B of Regulation (EU) No 283/2013 states that genotoxicity studies are required for exotoxins (using the purified chemical); this is not relevant to *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857). β-Exotoxins have been shown to be absent according to the US EPA data requirements (see Vol 4). Studies of genotoxicity using *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857) have not, therefore, been conducted. However, a study was already available in the original DAR.

***In vitro* studies**

A study of genotoxicity (bacterial reverse mutation), performed using a DMSO extract of XenTari® Technical Powder (ABG-6305) (*Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857)), was assessed in the EU Review and was presented in the DAR.

Information from the DAR, in a slightly different format:

Report:	IIM 5.3.5/01, Lawlor T (1997)
Title:	Mutagenicity test with XenTari Technical Powder (ABG-6305) in the *Salmonella – Escherichia coli*/mammalian- microsome reverse mutation assay
Document No:	18447-0-409
Guidelines:	US EPA Guideline 84-2
GLP:	Yes

Study design: Test material: XenTari Technical Powder ABG-6305 (Lot No.: 14455V9, total spore count: 6.2 x 10^{10} spores/g. A 20% (w/w) suspension of XenTari Technical Powder was prepared in DMSO, the suspension was sonicated for 15 minutes, mixed using a vortex for 3 minutes, and centrifuged (4,160 x g, 10 minutes). The supernatant was removed and the extract was filter sterilized.
All dose solutions were prepared from this supernatant.
Based on the results of initial dose range-finding studies, concentrations of 0 (solvent control) 10, 25, 50, 100, 150 and 200 µL/plate DMSO extract were selected for the mutagenicity assay, which was performed in triplicate. The tester strains *S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2uvrA were tested for mutagenicity in the presence and absence of metabolic activation (S9 mix) using the plate incorporation method.

Findings: No statistically significant increases in revertant colonies were noted for any of the test strains when exposed to a DMSO extract of XenTari® Technical Powder, both with and without metabolic activation.

Conclusion: The DAR concluded that, under the conditions of the study XenTari® Technical Powder (ABG-6305) did not cause a positive increase in the number of revertant colonies per plate when tested both with and without metabolic activation. It is further stated, however, that the study is inadequate for testing the potential genotoxic effect due to lack of characterisation of the material and an absence of appropriate positive controls.

B.6.1.2.4 Cell culture study

According to Part B of Regulation (EU) No 283/2013, a cell culture study is required only for intracellular replicating organisms and is therefore not relevant for *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857).

B.6.1.2.5 Information on short-term toxicity and pathogenicity

Supplementary studies on short-term toxicity, pathogenicity or infectiveness are not required for *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857). There is no evidence of toxicity, pathogenicity or infectiveness from the available standard acute studies. A number of studies are available, however, and are presented.

Health effects after repeated inhalatory exposure

No data are available for this endpoint. *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857) is shown to be of low acute toxicity and is not infectious or pathogenic by all routes investigated. Specific studies investigating toxicity and pathogenicity following short-term repeated dosing are therefore not scientifically justified. The DAR concluded, based on the low acute toxicity and absence of infectivity and pathogenicity of *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857) following acute exposure, together with the results of short-term exposure studies reported in the literature performed with other strains of *B. thuringiensis*, that further data to address this endpoint were not required. The DAR notes that short-term toxicity studies have been conducted in sheep and dogs using *Bacillus thuringiensis* ssp. *kurstaki* (in the DAR referred to as Hadley et al, 1987 and Pore, 1998, without further detailed evaluation) and in rats and dogs using *Bacillus thuringiensis* ssp. *Israelensis* (in the DAR referred to as Kandasamy, 1998 and Kumar, 2000, without further detailed evaluation). The sheep and dog studies were conducted using oral administration for 5 months and 90 days respectively and the sheep studies were conducted with two different strains of *Bacillus thuringiensis* ssp. *kurstaki*. The rat study was conducted by inhalation of *Bacillus thuringiensis* ssp. *israelensis* for 4 hours per day for 14 days. No significant treatment related effects were noted in any of these studies, thereby showing that short term exposure of a variety of animal species to *Bacillus thuringiensis* by the oral or inhalation route does not cause toxicity. The DAR further notes that *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857) is probably not absorbed following oral or intratracheal exposure. The absence of toxicity, infectivity or pathogenicity in an acute study using intravenous dosing (in which tissues were exposed to much higher levels of the microorganism than could be achieved in a short term toxicity study using inhalation exposure) is therefore significant.

Several papers on safety assessments of Cry protein were found in the literature search. Genetically modified maize, encoding *Bacillus thuringiensis* Cry1AB protein, fed to rats at levels of 11% and 33% in the diet for 90-days did not result in any effects, compared to control groups administered conventional maize for the same period (Hammond et al, 2006).

Administration of purified Bt protein Cry1Ab to male rats with experimentally-induced gastrointestinal impairment in a 4-week toxicity study resulted in reduced food consumption and weight gain, mild

changes in haematological and clinical chemistry and pathology indicative of intestinal ulceration and haemorrhage. Effects in the group without the experimentally-induced gastrointestinal impairment were limited to a slight but statistically significant reduction in serum AST activity, not considered to be clearly related to treatment and not of toxicological significance (Onose et al, 2008).

Report:	MMA 5.2.5.1/01; Hammond BG, Dudek R, Lemen JK & Nemeth MA (2006)
Title:	Results of a 90-day safety assurance study with rats fed grain from corn borer-protected corn
Reference:	Food and Chemical Toxicology 44: 1092–1099
Guidelines:	OECD 408
GLP:	Yes

Abstract: The toxicity of genetically modified (MON 810) maize, encoding *Bacillus thuringiensis* Cry1AB protein was investigated in rats following sub-chronic (90-day) dietary administration at levels of 11% and 33%. No effects were observed, compared to control groups administered conventional maize for the same period.

Material: Genetically modified (MON 810) maize, encoding *Bacillus thuringiensis* Cry1AB protein.

Methods: Groups of 20 Sprague-Dawley rats/sex (6 weeks old) were fed a diet containing MON 810 at levels of 11% or 33%. Control groups of rats were fed normal maize at the same inclusion levels. The parameters measured in this study were consistent with the those specified by the test guideline (OECD 408) and included bodyweight and weight gain; food consumption; haematological, clinical chemistry and urinalysis parameters; organ weights; gross necropsy and detailed histopathology.

Findings: No deaths occurred and there were no signs of toxicity. Bodyweights, weight gain and food consumption were comparable in all groups. There were no treatment-related effects on haematological, clinical chemistry or urinalysis parameters. Some statistically significant effects were seen on individual parameters, but were not of great magnitude, within the reference range and/or did not show a dose-response relationship. Organ weights were unaffected by treatment. Necropsy did not reveal and gross or microscopic findings considered to be related to treatment with MON 810.

Conclusion: Sub-chronic exposure of rats to diet containing genetically modified (MON 810) maize, encoding *Bacillus thuringiensis* Cry1AB protein was found to be without toxicologically relevant effects. This conclusion was also supported by the EFSA GMO Panel.

Report:	MMA 5.2.5.1/02; Onose J, Imai T, Hasumura M, Ueda M, Ozeki Y & Hirose M (2008)

Title:	Evaluation of subchronic toxicity of dietary administered Cry1Ab protein from *Bacillus thuringiensis* var. *Kurustaki* HD-1 in F344 male rats with chemically induced gastrointestinal impairment.
Reference:	Food and Chemical Toxicology 46: 2184–2189
Guidelines:	None
GLP:	No

Abstract: Purified Bt protein Cry1Ab was administered to male F344 rats in a 4-week toxicity study. The effects of experimentally-induced gastrointestinal impairment on the toxicity of Bt protein Cry1Ab were also investigated. The gastrointestinal impairment groups showed reduced food consumption and weight gain, mild changes in haematological and clinical chemistry and pathology indicative of intestinal ulceration and haemorrhage. Effects in the group administered Bt protein Cry1Ab alone were limited to a slight but statistically significant reduction in serum AST activity, not considered to be clearly related to treatment and not of toxicological significance.

Material: Purified Bt protein Cry1Ab from *B. thuringiensis* var. *Kurustaki* HD-1.

Methods: A groups of eight male F344 rats (6 weeks old) was administered purified Bt protein Cry1Ab in the diet at a concentration of 10 ppm during the second and fourth weeks of a four-week study. This group was given basal diet during the first and third weeks of the study. A control group was administered basal diet throughout the study. A further group of rats was treated with indomethacin (80 ppm in the diet) during the first and third weeks of the study and with basal diet and twice daily intraperitoneal injections of famotidine (30 mg/kg bw) during the second and fourth weeks of the study, as a model of gastrointestinal impairment (reduced gastric acid secretion and small intestine inflammation). A final group was administered purified Bt protein Cry1Ab in the diet at a concentration of 10 ppm during the second and four the weeks of the study, in conjunction with the induction of gastrointestinal impairment as described for the previous group. Rats were observed daily for mortality and signs of toxicity; bodyweights and food consumption were measured weekly. Terminal blood samples were taken for the assessment of haematological and clinical chemistry parameters. Gross necropsy was performed on all rats; weights of the brain, thymus, lungs, heart, spleen, liver, adrenals, kidneys and testes were recorded. A large number of tissues from each rat were subject to histopathological investigation.

Findings: No deaths occurred and there were no signs of toxicity. Significant reductions in food consumption and weight gain were seen for both gastrointestinal impairment groups. Haematology revealed a slight reduction in erythrocyte count and a slight increase in platelet count in both gastrointestinal impairment groups; findings did not attain statistical significance. Clinical chemistry showed changes (increased A:G ratio, reduced triglyceride concentration and increased cholesterol concentration) in both gastrointestinal impairment groups. Serum AST activity was significantly reduced in all treated groups. Abs organ weights were unaffected by treatment; a small number of statistically significant changes in relative organ weights seen in both gastrointestinal impairment groups are secondary to the lower terminal bodyweights in the groups. Gross necropsy showed adhesions and granulomatous nodules in the small intestine of rats in both of the gastrointestinal impairment groups. Findings were associated with microscopic observations of intestinal

ulceration, severe extramedullary haematopoiesis in the spleen and increased bone marrow cellularity.

Table 5.2.5-01 Summary of clinical chemistry findings

Parameter	Control	Cry1Ab	GI impaired	GI impaired + Cry1Ab
TP (g/dL)	6.1	6.1	5.9	5.9
Albumin (g/dL)	3.8	3..8	3.9	4.0
A:G	1.7	1.9	2.0**	2.1**
TG (mg/dL)	96.1	88.5	66.9*	68.8*
Cholesterol (mg/dL)	63.8	64.6	74.5*	70.0*
BUN (mg/dL)	19.7	19.6	20.6	19.0
AST (IU/L)	95.1	67.6**	78.1**	63.1**
ALT (IU/L)	37.4	38.3	40.1	40.1
ALP (IU/L)	877	932	919	927

**significantly different to controls ($p<0.05$); **$p<0.01$*

Discussion: Administration of purified Bt protein Cry1Ab to rats under the conditions of this study was not associated with any effect with the exception of a reduced serum AST activity. The toxicological significance of this finding is unclear, as organ damage is associated with an increase in serum AST activity. Similar effects were seen in the other treated groups and no reference is made to a normal background range. This finding is therefore considered likely to be incidental and/or not of toxicological significance.

Conclusion: Administration of purified Bt protein Cry1Ab to rats under the conditions of this study was not associated with any toxicologically significant effect

B.6.1.2.6 Proposed treatment: first aid measures, medical treatment

Bacillus thuringiensis ssp. *aizawai* (strain ABTS 1857) is not toxic, infective or pathogenic. First aid measures and a specific therapeutic regimen cannot therefore be recommended.

For accidental eye exposure: Remove from source of exposure. Flush the affected eye(s) with copious amounts of water. If irritation persists, seek medical attention. Provide symptomatic/supportive care as necessary.

For accidental skin exposure: Remove from source of exposure. Wash the affected area with water. If irritation persists, seek medical attention. Provide symptomatic/supportive care as necessary.

For accidental ingestion: Remove from source of exposure. If signs of toxicity occur, seek medical attention. Provide symptomatic/supportive care as necessary.

For accidental inhalation: Remove from source of exposure. If irritation or signs of toxicity occur, seek medical attention. Provide symptomatic/supportive care as necessary.

Treatment: Supportive therapy, symptomatic treatment.

B.6.1.3 Toxicity studies on metabolites and relevant impurities

There are no studies performed or submitted.

B.6.1.4 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

B.6.2 Tier II

B.6.2.1 Specific toxicity, pathogenicity and infectiveness studies

Supplementary studies on specific toxicity, pathogenicity or infectiveness are not required for *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857). There is no evidence of toxicity, pathogenicity or infectiveness from the available standard studies. Although the following studies are not very relevant they are summarised here.

Report:	MMA 5.3/01, Lemos AJJM, Wanderley-Teixera V, Teixera AAC, Silva FdCA, Oliveira JV , & Siquiera HAA (2011)
Title:	Response of blastocyst–endometrium interactions in albino rats to sublethal doses of biological and synthetic insecticides
Reference:	Food & Chemical Pathology 49:2541-2547
Guidelines:	None
GLP:	No

Abstract: The effects of XenTari® WG on implantation numbers and uterus histopathology were investigated in the rat following the gavage administration of XenTari® WG on Gestation Days 0-7. Administration of XenTari® WG at a dose level of 3700 mg/kg bw/d caused a reduction in implantation numbers and marked histopathological changes. The authors suggest that the changes in the uterine decidua impeded the adherence and fixation of the embryo, resulting in a reduction in the number of implantation sites

Material: XenTari® WG, containing *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857).

Methods: Groups of five pregnant female Wistar rats (90 days old, ~200 g bodyweight) were gavaged with XenTari® WG at dose levels of 0 (administered water), 185, 1850 or 3700 mg/kg bw to Gestation Day 7. Animals were terminated and the uteri assessed for the presence of implantation sites following fixation in Bouin's solution; implantation sites were also analysed morphometrically for the quantification of vascularisation. The implantation sites were subsequently assessed histopathologically following staining with haematoxylin & eosin or with Mallory's trichrome for the assessment of connective tissue.

Findings: Maternal bodyweight is reported to have been unaffected by treatment; other evidence of maternal toxicity is not reported. A significant reduction in the number of implantation sites was seen in rats administered 3700 mg/kg bw/d, compared to controls. Histologically, implantation sites in the control group were well developed and were composed of trophoblasts (some with mitotic activity), polyploid cytotrophoblasts and showed extensive vascularisation. Significant histological changes were observed in the group administered 3700 mg/kg bw/d. Findings were characterised by the presence of vacuolated trophoblastic cells, sparse cytotrophoblasts, large amounts of blood vessels (some with accentuated leukocyte infiltration), decidual degeneration and the presence of blood in the uterine lumen. Mallory's trichrome staining produced a markedly more intense reaction in the decidua of rats administered 3700 mg/kg bw/d.

Table 5.3-01 Summary of findings

Dose level (mg/kg bw/d)	**0**	**185**	**1850**	**3700**
Pups (#)	14.0	11.2	11.2	9.4

Discussion: The authors suggest that the changes in the decidua impeded the adherence and fixation of the embryo, resulting in a reduction in the number of implantation sites (there was no evidence of post-implantation loss). Effects were, however, seen at a dose level significantly exceeding the regulatory limit dose of 1000 mg/kg bw/d and are therefore not considered to be of clear toxicological significance. The authors also investigated the effects of a deltamethrin product, and report a significant reduction in implantation numbers and very similar histopathological findings at a dose level of 4 mg/kg bw/d, which (based on regulatory toxicity studies performed with deltamethrin) would not be expected to show any reproductive toxicity. The findings reported for this study are therefore considered to be questionable.

Conclusion: The effects of XenTari® WG on implantation numbers and gravid uterine histopathology reported in this published study are not considered to be of clear relevance to the human risk assessment. Effects were reported with the commercial product only at dose levels exceeding the regulatory limit dose. It is also notable that very similar effects are also reported for deltamethrin, and are not characteristic of the toxicity of this well-investigated insecticide.

Report:	MMA 5.3/02, Lemos AJJM, Siquiera HAA, Wanderley-Teixera V, Maia FCL, Teixera AAC, Silva EJ & Oliveira JV (2013)
Title:	Effect of sub-lethal doses of *Bacillus thuringiensis* subsp. *aizawai* and deltamethrin with regard to fertility and organ toxicity in pregnant albino rats
Reference:	Experimental and Toxicologic Pathology 65:489-495.
Guidelines:	None
GLP:	No

Abstract: The effects XenTari® WG on organ pathology and embryofoetal development were investigated in the rat following the gavage administration of XenTari® WG on Gestation Days 0-7 or throughout gestation. Administration of XenTari® WG at a dose

level of 1850 or 3700 mg/kg bw/d to GD7 resulted in histopathological effects on the kidneys, liver and lungs and also resulted in a reduction in the number of pups born.

Material: XenTari® WG, containing *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857).

Methods: Groups of ten pregnant female Wistar rats (90 days old, ~200 g bodyweight) were gavaged with XenTari® WG to Gestation Day 7 (5 females) or throughout pregnancy at dose levels of 0 (administered water), 185, 1850 or 3700 mg/kg bw/d. Rats terminated at GD7 were investigated histopathologically for effects on the liver, kidney and lungs. Rats treated throughout gestation were allowed to litter; the resulting offspring were counted weighed and measured and assessed for external malformations.

Findings: Administration of XenTari® WG at a dose level of 3700 mg/kg bw/d to GD7 resulted in histopathological effects on the kidney (necrosis and vacuolar degeneration of the convoluted tubules and collecting ducts; proliferative glomerulonephritis and a significant reduction in Bowman's space; accentuated hypertrophy, hyperplasia and stratification of the tubular epithelium. Effects were also seen in the liver of rats administered XenTari® WG at dose levels of 1850 or 3700 mg/kg bw/d; findings were characterised by a slight increase in sinusoidal space, coagulative nodular focal necrosis with an 'intense' Kupffer cell reaction. In the lungs, inflammatory reactions characterised by the presence of macrophages, polymorphonuclear cells, purulent bronchiolitis, peribronchiolitis and perivasculitis with macrophage and lymphocyte involvement.

Administration of XenTari® WG at dose levels of 1850 and 3700 mg/kg bw/d throughout gestation resulted in a marked reduction in the number of pups. There was no effect of treatment on pup weight, pup length or on the incidence of external malformations.

Table 5.3-02 Summary of findings

Dose level (mg/kg bw/d)	0	185	1850	3700
Pups (#)	12.0	11.6	8.6	6.2
Pup length[1]	6.17	5.92	6.30	6.16
Pup weight[1]	6.14	5.57	5.80	5.97

[1]*unit of measurement not reported*

Discussion: This study reports marked effects of XenTari® WG on the kidneys, liver and lungs, albeit only at dose levels exceeding the regulatory limit dose of 1000 mg/kg bw/d.

It is notable that the study was performed with the commercial product rather than the active substance; therefore the reported effects may be due to the active substance and/or co-formulants. The study is not reported in great detail; notably there is no information on general maternal toxicity (clinical signs, food consumption and bodyweight) which are essential in putting the reported effects in context. It is also unclear what the control group was exposed to. The active substance and some of the product coformulants have nutritional value to the rat and, particularly when administered at very high dose levels as was the case in this study, are likely to have physiological effects. The effects of XenTari® WG on the lungs are severe, and are considered most likely to be a consequence of dosing accident than to be due to a systemic effect of the test material. It is notable that the study also reports marked effects in the lung (thick-

ened septa indicating moderate interstitial pneumonia, an inflammatory reaction around the bronchioles, peribronchiolar infiltrate and multi-focal pneumonia) following the administration of relatively low dose levels of deltamethrin. Deltamethrin has been extensively investigated in regulatory toxicology studies and is not associated with lung toxicity. This study also reports effects of deltamethrin on the liver and kidney, also not recognised as targets of toxicity in regulatory studies. The marked effects of XenTari® WG on pup number are associated with maternal toxicity (effects on the lungs, liver and kidneys); however other important aspects of maternal toxicity were not reported. As indicated above, it would seem likely that maternal animals were in poor condition due to dosing accidents. The study also reports developmental toxicity (a marked reduction in pup number, without effects on pup length or weight) at relatively low dose levels of deltamethrin. Deltamethrin has been investigated for developmental and reproductive toxicity in regulatory studies and shown to be without significant effects. The findings reported in this study are therefore questionable.

Conclusion: The effects of XenTari® WG on organ pathology and reproductive toxicity reported in this published study are not considered to be of clear relevance to the human risk assessment. Effects were reported with the commercial product only at dose levels exceeding the regulatory limit dose; the reliability of the study is also compromised by reporting deficiencies and the likelihood of dosing accidents. It is also notable that very similar effects on organ pathology and reproductive toxicity are also reported of deltamethrin, and are not characteristic of the toxicity of this well-investigated insecticide.

B.6.2.2 *In vivo* studies in somatic cells

No data are available and none are required. Part B of Regulation (EU) No 283/2013 states that *in vivo* genotoxicity studies in somatic cells are required when positive results are obtained in studies of genotoxicity *in vitro*. In the absence of any indication of genotoxicity for *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857), this data requirement is not relevant and no additional studies are required.

Although not relevant the following published studies of genotoxicity have, however, been identified by the literature search, although 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:

Report:	MMA 5.4/01; Freire IS, Miranda-Vilela AL, Barbosa LCP, Martins ES, Monnerat RG & Grisolia CK (2014a).
Title:	Evaluation of Cytotoxicity, Genotoxicity and Hematotoxicity of the Recombinant Spore-Crystal Complexes Cry1Ia, Cry10Aa and Cry1Ba6 from *Bacillus thuringiensis* in Swiss Mice
Reference:	Toxins 6: 2872-2885
Guidelines:	None
GLP:	No

Abstract: The genotoxicity of recombinant Bt strains expressing the crystal endotoxins, BtCry1Ia, BtCry10Aa or BtCry1Ba6 following oral administration was investigated in a mouse bone marrow micronucleus assay. No evidence of genotoxicity was seen under the conditions of this assay.

Material: The nature of the test material is unclear from the details presented in the paper, and is described as 'recombinant spore crystals'. It would appear that the materials tested were recombinant Bt strains engineered to produce the crystal endotoxins, BtCry1Ia, BtCry10Aa or BtCry1Ba6.

Methods: Groups of six male Swiss albino mice were exposed orally for 72 hours to the Bt spore crystal solutions prepared in 0.9% saline solution and administered at three different dose levels:

Cry10Aa: 1 x10^9 spores/kg, 5 x10^9 spores/kg and 1 x10^{10} spores/kg;

Cry1Ba6: 4 × 10^8 spores/kg, 2 x10^9 spores/kg and 4 x10^9 spores/kg;

CryI1a: 4 × 10^8 spores/kg, 2 x10^9 spores/kg and 4 x10^9 spores/kg.

Animals were sacrificed and blood samples were taken at 72 hours by cardiac puncture. Peripheral blood smears were assessed for the presence of micronuclei following staining with acridine orange. 3000 normochromatic erythrocytes (NCEs) and polychromatic erythrocytes (PCEs) were examined for each mouse; the number of micronucleated PCEs and the PCE:NCE ratio were recorded.

Findings: There was no evidence for a treatment-related increase in the incidence of micronucleated PCEs in any treated group of mice. The proportion of PCEs was significantly decreased and the proportion of NCEs correspondingly increased in mice administered Cry10Aa at the lowest dose level; the absence of a dose-response relationship suggests that this finding is not treatment-related.

Table MMA 5.3.2.1-01 Micronucleus frequency (de Souza *et al*., 2014)

Exposure		Mn-PCEs (/3000 PCEs)
Control	-	0.67
Cry10Aa	1 x10^9 spores/kg	0.17
	5 x10^9 spores/kg	0.67
	1 x10^{10} spores/kg	0.17
Cry1Ba6	4 x10^8 spores/kg	0.3
	2 x10^9 spores/kg	0.17
	4 x10^9 spores/kg	0.33
Cr1IA	4 x10^8 spores/kg	0.67
	2 x10^9 spores/kg	0.83
	4 x10^9 spores/kg	0.67

Conclusion: No evidence of genotoxicity was seen under the conditions of this study. None of the Cry

toxins tested in this study are produced by Bta (Strain ABTS 1857); the study is therefore of unclear relevance.

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Report:	MMA 5.4/03; Mezzomo BP, Miranda-Vilela AL, de Souza Freire I, Barbosa LCP, Portilho FA & Grisolia CK (2015).
Title:	Hematotoxicity of *Bacillus thuringiensis* as Spore-crystal Strains Cry1Aa, Cry1Ab, Cry1Ac or Cry2Aa in Swiss Albino Mice.
Reference:	Journal of Hematology & Thromboembolic Diseases 1(1)
Guidelines:	None
GLP:	No

Abstract: Groups of mice were gavaged with single doses of spore crystals Cry1Aa, Cry1Ab, Cry1Ac and Cry2Aa from *B. thuringiensis* var. *kurstaki* at dose levels of 27, 136 or 270 mg/kg bw and terminated at 24 hours, 72 hours and 7 days. Groups of mice were also gavaged with binary combination of spore crystals at a dose level of 27 mg/kg bw and terminated at 24 hours. Terminal blood samples were taken for the detailed assessment of haematological parameters and bone marrow assessed for the formation of micronuclei. Effects of treatment were seen on haematological parameters; no effects of treatment were seen on micronucleus frequency. The authors conclude that the results of this study show evidence of erythroid haematotoxicity but no genotoxicity; however the study is not considered to be reliable for a number of reasons.

Material: The nature of the test material is unclear from the details presented in the paper, and is described as 'genetically modified strains of Bt spore crystals'. It is assumed that the material tested was actually recombinant Bt strains engineered to produce the crystal endotoxins Cry1Aa, Cry1Ab, Cry1Ac or Cry2Aa.

Methods: Groups of three Swiss mice/sex (aged ~90 days) were administered single gavage doses of the Cry1Aa, Cry1Ab, Cry1Ac and Cry2Aa spore crystals (suspended in distilled water) at dose levels of 27, 136 or 270 mg/kg bw. Groups of mice were terminated at 24 hours, 72 hours or 7 days following exposure. Combinations of the spore crystals (Cry1Aa/1Ab, Cry1Aa/1Ac, Cry1Aa/2Aa, Cry1Ab/1Ac, Cry1Ab/2Aa, Cry1Ac/2Aa) were also administered as a single gavage dose at 27 mg/kg bw and mice terminated at 24 hours following dosing. Control mice received water or cyclophosphamide (27 mg/kg bw). Terminal blood samples were taken for the detailed assessment of haematological parameters and bone marrow assessed for the formation of micronuclei.

Findings: The authors present statistically significant effects of treatment on haematological parameters; however the results are not considered to be reliable based on the small group size (3 in stead of 5) containing mice of both sexes, the lack of reference to a background range and, in many cases, the absence of a dose-response relationship. Administration of the spore crystals did not result in any statistically significant increase in the frequency of micronucleated polychromatic or normochromatic erythrocytes, in fact markedly reduced frequencies of micronucleated PCEs were reported for some treated groups. It is notable that the positive control did not cause any signifi-

cant effect on micronucleus frequency. Slight (but statistically significant) reductions in the proportion of PCEs were seen for the positive control and some treated groups.

Table MMA 5.3.2.1-02 Micronucleus frequency

Dose group		MnNCE[1]	MnPCE[1]	% PCE
Negative control		2.00	2.50	52.61
Cry1Aa	**27 mg/kg bw**	0.40	3.00	48.55
	136 mg/kg bw	0.43	2.29	49.28*
	270 mg/kg bw	1.00	3.67	52.90
Cry1Ab	**27 mg/kg bw**	1.67	4.67	45.92**
	136 mg/kg bw	1.50	3.33	47.78*
	270 mg/kg bw	1.00	2.17	52.91
Cry1Ac	**27 mg/kg bw**	1.33	3..33	49.49
	136 mg/kg bw	2.50	5.17	449.2**
	270 mg/kg bw	2.17	4.00	48.69
Cry2Aa	**27 mg/kg bw**	1.00	0.83	47.06**
	136 mg/kg bw	0.50	2.00	47.93
	270 mg/kg bw	0.17	0.50	48.63
Cry1Aa/1Ab	**27 mg/kg bw**	0.50	0.67	45.02
Cry1Aa/1Ac	**27 mg/kg bw**	0.33	0.83	46.72
Cry1Aa/2Aa	**27 mg/kg bw**	0.17	0.83	48.88*
Cry1Ab/1Ac	**27 mg/kg bw**	0.50	1.50	51.46
Cry1Ab/2Aa	**27 mg/kg bw**	1.00	2.83	466.50*
Cry1Ac/2Aa	**27 mg/kg bw**	0.50	1.83	46.51*
Positive control		2.17	3.50	45.97**

[1]*frequency: units not reported*

**significantly different to negative control (p<0.05); **p<0.01*

Discussion: This study is published in the Journal of Hematology & Thromboembolic Diseases, which is published by the OMICS Publishing Group. This group is widely regarded to be predatory (i.e. is an exploitative open-access publishing business model that involves charging publication fees to authors without providing the editorial and publishing services associated with legitimate journals). The validity of the peer review by journals of the OMICS Publishing Group has been questioned by academics and by the US Government. Journals of the OMICS Publishing Group are not accepted by the US NIH for listing in PubMed Central. Even discounting concerns over the validity of the journal, the results presented in this paper cannot be reliably interpreted due to deficiencies in methodology, performance and reporting. It is further noted

that apparently the same study was previously published in a reputable journal[25], but was subsequently withdrawn.

Conclusion: The authors conclude that the results of this study show evidence of haematotoxicity but no genotoxicity in the mouse following a single gavage administration of spore-crystals Cry1Aa, Cry1Ab, Cry1Ac and Cry2Aa from *B. thuringiensis* var. *kurstaki*. The authors suggest that the haematotoxicity seen in this study was more marked on cells of the erythroid lineage, indicating an interaction between the crystal proteins and the cell membrane. The study cannot, however, be considered to be reliable.

Note RMS: Could applicant explain why the study performed by Mezzomo 2015 was withdrawn?

Report:	MMA 5.4/03; Mezzomo BP, Miranda-Vilela AL, Barbosa LCP, Albernaz VL & Grisolia CK (2015).
Title:	Hematotoxicity and Genotoxicity Evaluations in Swiss Mice Intraperitoneally Exposed to *Bacillus thuringiensis* (var *kurstaki)* Spore Crystals Genetically Modified to Express Individually Cry1Aa, Cry1Ab, Cry1Ac, or Cry2Aa
Reference:	Environmental Toxicology 31(8):970-978.
Guidelines:	None
GLP:	No

Abstract: The haematological effects and the induction of bone marrow micronucleus formation was investigated in Swiss mice following the administration of a single intraperitoneal injection of *Bt* spore crystals Cry1Aa, Cry1Ab, Cry1Ac or Cry2Aa. Haematological effects were observed in the majority of all treated groups and the proportion of micronucleated NCEs and PCEs were also significantly increased in the majority of treated groups. Findings suggest a haematotoxic and potentially genotoxic effect of *Bt* spore crystals when administered at high intraperitoneal doses.

Material: The nature of the test material is unclear from the details presented in the paper, and is described as 'genetically modified strains of Bt spore crystals'. It is assumed that the material tested was actually recombinant Bt strains engineered to produce the crystal endotoxins Cry1Aa, Cry1Ab, Cry1Ac or Cry2Aa.

Methods: Groups of three Swiss mice/sex (10-12 weeks old) were administered a single intraperitoneal dose of the lyophilised *Bt* spore crystals Cry1Aa, Cry1Ab, Cry1Ac or Cry2Aa. Based on the results of a pilot study, all spore crystals were administered at a dose level of 27 mg/kg bw; Cry1Aa, 1Ab and 2Aa were also administered at 136 and 270 mg/kg bw. Control groups received water (negative controls) or cyclophosphamide (positive controls), also by intraperitoneal injection. All mice were terminated at 24 hours after dosing. Blood samples were taken for haematological assessment; bone marrow cells were harvested and assessed for the presence of micronuclei.

[25] Mezzomo BP. Miranda-Vilela AL, de Souza Freire I, Barbosa LC, Portilho FA, LacavaZG & Grisiola CK (2012). Effects of oral administration of *Bacillus thuringiensis* as spore-crystal strains Cry1Aa, Cry1Ab, Cry1Ac or Cry2Aa on hematologic and genotoxic endpoints in Swiss Albino Mice. Food & Chemical Toxicology 2012 Nov 9. pii: S0278-6915(12)00777-6. doi: 10.1016/j.fct.2012.10.032. [Epub ahead of print]

Findings: Mice administered Cry1Ac (27 mg/kg bw) showed a significant reduction in MCHC compared to controls; however the value was within the reference range. Hypochromia and anisocytosis were also increased in this group. Mice administered Cry1Ab (270 mg/kg bw) also showed a significant decrease in MCHC. A significantly reduced lymphocyte count was observed after treatment with Cry1Aa (270 mg/kg bw), Cry1Ab (136 and 270 mg/kg bw and Cry2Aa (136 and 270 mg/kg bw); all values were below the reference range. Cry1Ab (270 mg/kg bw) also caused a significant increase in neutrophils + monocytes. Although statistically non-significant compared to the negative control, Cry1Aa (136 mg/kg bw) caused a reduction below the reference range for the total white blood cell (WBC) count, due mainly to lymphocytes and neutrophils + monocytes. This finding was associated with an increased eosinophil count. The majority of treatments also increased platelet counts and/or platelet distribution width values.

A significant increase in the proportion of micronucleated NCEs and PCEs was seen for the majority of treatments; a significant reduction in the proportion of PCEs was also observed.

Table MMA 5.3.2.1-03 Micronucleus frequency

Dose group		MnNCE[1]	MnPCE[1]	% PCE
Negative control		0.17	2.33	70.13
Cry1Aa	**27 mg/kg bw**	2.17**	3.33	41.69**
	136 mg/kg bw	3.67**	5.17*	49.32**
	270 mg/kg bw	4.20**	7.00**	39.68**
Cry1Ab	**27 mg/kg bw**	3.57**	8.71**	56.63
	136 mg/kg bw	6.50**	12.17**	51.91**
	270 mg/kg bw	6.17**	9.67*	46.28**
Cry1Ac	**27 mg/kg bw**	2.50**	5.67**	51.36**
Cry2Aa	**27 mg/kg bw**	3.83**	4.33	42.66**
	136 mg/kg bw	4.67**	5.50**	37.39**
	270 mg/kg bw	2.33**	5.00	34.71**
Positive control		14.33**	26.83**	66.56

[1]*frequency: units not reported*

**significantly different to negative control ($p<0.05$); **$p<0.01$*

Discussion: The results of this study show a marked increase in the frequency of micronucleated polychromatic and normochromatic erythrocytes following the administration of single intraperitoneal doses of the lyophilised *Bt* spore crystals Cry1Aa, Cry1Ab, Cry1Ac or Cry2Aa. Findings were associated with a marked reduction in the proportion of PCEs, indicating significant direct bone marrow toxicity, as well as effects on haematological parameters. The dose route used in this study is non-physiological and cannot be considered to be representative of exposure in any normal situation. Furthermore, it is likely that the intraperitoneal injection of large quantities of bacterial protein would result in other marked physiological effects (notably hyperthermia) which are known to increase the incidence of micronuclei. The increases in micronucleus frequency were also associated with a high degree of bone marrow toxicity, which is not seen in any other study using a representative route of exposure. Although published in a reputable journal, it is noted that this study is reported by the authors of the previous paper and is therefore questionable in terms of reliability.

Conclusion: The findings of this study do not indicate a genotoxic hazard of Bt spore crystals following normal (physiological) routes of exposure, and are not therefore considered to be relevant to the risk assessment.

B.6.2.3 Genotoxicity – *In vivo* studies in germ cells

No data are available and none are required. Part B of Regulation (EU) No 283/2013 states that *in vivo* genotoxicity studies in germ cells are required when positive results are obtained in studies of genotoxicity in somatic cells in vivo. In the absence of any indication of genotoxicity for *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857), this data requirement is not relevant and no additional studies are required.

B.6.2.4 Summary and conclusions of Tier II studies

No relevant studies to be used for the risk assessment.

B.6.3 Summary of mammalian toxicity, pathogenicity and effectiveness and overall evaluation of the active micro-organism

Mode of action and insect specificity

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 products depends on the type of Cry-proteins they produce. 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.

B. thuringiensis and food poisoning

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* bio-opesticides (*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).

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.

Conclusion

Based on the low toxicity, lack of pathogenicity and infectiveness and the negligible potential to cause food poisoning, it can be concluded based on the available data that *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS 1857) can be used without risk to human health.

B.6.4 References relied on

A literature search was performed (Duffy, 2016).

The report summarises an evaluation of several literature searches performed according to the EFSA document; Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009. EFSA Journal 2011; 9(2) : 2092 for public domain literature on *Bacillus thuringiensis aizawai* ABTS-1857 (Bta). Results of the search are provided below.

The search strategy was based on a single concept search. For details regarding the search strategy and the results obtained, please refer to section 5 of the Literature Review Report.

Relevant publications are those showing new/unknown effects or information potentially contradictory to the regulatory data package for the active substance, on human health and/or the environment, which could impact the endpoints or the risk assessment parameters.

Studies providing information that supports the existing regulatory data package were considered as non-relevant.

The relevance criteria are presented in Table 1.

Note RMS:

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 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).

Table 1: Relevance criteria

Data requirement(s) indicated by the correspondent OECD data point number(s)	Criteria for relevance
Analytical methods (OECD code: IIA 4)	1. Well described method 2. SANCO/825/00 rev. 8.1.guideline referenced
Toxicological and metabolism studies (OECD code: IIA 5.1 to 5.7)	1. Well-defined test material (including its purity and impurity profile). 2. Relevant test species (to the mammalian toxicological assessment -preferred species are rodents - rats and mice, the dog is the preferred non-rodent species). 3. Number of animals per group sufficient to establish a statistical significance. 4. Several dose levels tested (at least 3), preferably including a negative control, to establish a dose-response. 5. Relevant route of administration in terms of risk assessment (oral, dermal or by inhalation). 6. Description of the observations, examinations, analysis performed, or necropsy. 7. Well described test methodology – appropriate guideline referenced 8. In addition: studies which may be helpful for the interpretation of other studies present in the dossier, but do not fit under a specific toxicological endpoint.
Residues (OECD code: IIA 6)	1. Well-defined test material (including its purity and impurity profile). 2. Any information on residues in crops relevant to the EU 3. Well described methodology and results
Fate and behaviour in the environment (OECD code: IIA 7)	1. Any information that could affect the environmental parameters, degradation profile or endpoints used in the risk assessment 2. Any environmental monitoring information relevant to the usage pattern. 3. Well defined test material (including its purity and impurity profile). 4. Well described test methodology – appropriate guideline referenced
Ecotoxicological studies (OECD code: IIA 8)	1. Well defined test material (including its purity and impurity profile). 2. Relevant test species 3. Number of animals per group sufficient to establish a statistical significance. 4. Several dose levels tested (at least 3), preferably including a negative control, to establish a dose-response. 5. Well described test methodology – appropriate guideline referenced 6. Description of the observations, mortality etc, and chemical analysis performed. 7. In addition: studies which may be helpful for the interpretation of other studies present in the dossier, but do not fit under a specific ecotoxicological endpoint.

Details of the databases searched, justification for selection etc is provided below in Table 2.

No searches apart from bibliographic databases were undertaken.

Table 2: Reporting of the search process for scientific peer-reviewed open literature in bibliographic databases

Data requirement (s) captured in the search	**Details of the searches**				
Bacillus thuringiensis aizawai ABTS-1857 (Bta) Covers all data requirements	**BIOSIS**	**Toxcenter**	**Medline**	**CAplus**	**CABA**
	Justification for choosing the source: BIOSIS Previews® is the largest and most comprehensive life science database in the world. Amongst others subject coverage includes Agriculture, Biochemistry, Biophysics, Botany, Environmental Biology, Physiology, Toxicology. Sources include periodicals, journals, conference proceedings, reviews, reports, patents, and short communications. Nearly 6,000 life source journals, 1,500 international meetings as well as review articles, books, and monographs are reviewed for inclusion. Bibliographic information, indexing terms, abstracts, and CAS Registry Numbers are all searchable.	Toxicology Center covers the pharmacological, biochemical, physiological, and toxicological effects of drugs and other chemicals. TOXCENTER is composed of the following subfiles: BIOSIS (1969 to date), CAplus (1907 to date), IPA (1970 to date), and MEDLINE (1953 to date). Sources include abstracts, books and book chapters, bulletins, conference proceedings, journal articles, letters, meetings, monographs, notes, papers, patents, presentations, research and project summaries, reviews, technical reports, theses, translations, unpublished material, web reprints. Records contain bibliographic data, abstracts, indexing terms, chemical names, and CAS Registry Numbers	MEDLINE is the U.S. National Library of Medicine® (NLM) premier bibliographic database that contains more than 23 million references to journal articles in life sciences with a concentration on biomedicine. A distinctive feature of MEDLINE is that the records are indexed with NLM Medical Subject Headings (MeSH®). MEDLINE is the online counterpart to MEDLARS® (MEDical Literature Analysis and Retrieval System) that originated in 1964.	Chemical Abstracts Plus covers worldwide literature from all areas of chemistry, biochemistry, chemical engineering, and related sciences. Since October 1994 all articles from more than 1,600 key chemical journals are added including citations for documents not covered by CA. Coverage includes applied, macromolecular, organic, physical, inorganic, and analytical chemistry. Current sources include over 8,000 journals, patents and patent families from 38 national patent offices and 2 international patent organizations, technical reports, books, conference proceedings, dissertations, product reviews, bibliographic items, book reviews, and meeting abstracts. Electronic-only journals and Web preprints are also covered.	The CAB Abstracts database covers worldwide literature from all areas of agriculture and related sciences including biotechnology, forestry, and veterinary medicine. Sources for CABA include journals, books, reports, published theses, conference proceedings, and patents. Bibliographic information, indexing terms, abstracts, and CAS Registry Numbers are searchable.
	Date of the search: August 2016				
	Date span of the search: 1 January 2005 – 23 August 2016 (BTa)				
	Date of the latest database update included in the search: At 02/2016 24.8 million records were available, the database is updated weekly.	**Date of the latest database update included in the search:** At 01/2016 12.2 million records were available, the database is updated weekly.	**Date of the latest database update included in the search:** At 01/2016 25.3 million records were available, updated daily.	**Date of the latest database update included in the search:** At 01/2016 8 million records were available, updated weekly	**Date of the latest database update included in the search:** At 01/2016 8 million records were available, updated weekly

	Search strategies used for this data requirement
	Terms searched:

	"*Bacillus thuringiensis* AND aizawai" – this search term was applied to each of the search terms listed below by scientific area using the 'AND' operator (for example "Bacillus thuringiensis AND aizawai AND growth").						
	Biological Properties:						
	growth	spore coat	hydrophobicity	adherence	food poisoning		
	Human Toxicology:						
	?toxi?	?toxi? and mammal	human	worker	clinical	occup?	adverse
	sensitis?	sensitiz?	allergen	hypersens?	infect?	infect? AND mammal?	pathogen?
	pathogen? AND mammal		immunocomp	genotox?			
	Residues:						
	residu?	food					
	Environmental Fate:						
	distribution	soil	soil and europ?	water	water and europ?	air	
	Ecotoxicology:						
	non-target	fish or lepomis? or oncorhynchus or salmonidae or pimephales or cyprinid? or minnow or carp or zebraf? or goldfish? daphni? or asellus or chironom? or cloeon? or hyalella or 'aquatic invertebrate'				alga? or selenastrum? or scenedesmus? or chlorella? or skeletonema? or navicula? or anabaena?	
	(nontarget? and plant) and (lemna? or chara? or elodea? or myriophyllum? or glyceria? or duckweed? or pondweed)			arthro? or lacewing? or hoverfly? or ladybird# or syrphid? or phytoseiulus? or aphidius? or typhlodromus?			ecotox?
	adverse	pathogen?	enviro?	?toxi? and bird? or avian or mallard? or quail or bobwhite or repro? or oral or chronic			
	honeybee# or bumblebee# or apis or bombus? or bee###		honeybee and contact or oral or larvae or feed?	earthworm? or eisenia? or lumbricus or allobophora? or dendrobaena? or aporrectodea? or dendrodrilus? or hypoaspis? or collembola? or springtail or terrestrial			
	staphylinid? or coccinel? or pardosa? or orius? or bembidion? or hymenopt? or chrysopid?				bird? or avian or mallard? or quail or bobwhite or repro? or oral or chronic		
	Efficacy:						
	n/a						
	Resistance:						
	n/a						
	Total number of summary records retrieved: 1771						
						Total number of summary records retrieved after removing duplicates	**N = 519**

In total, 519 summary records were retrieved from bibliographic databases and were screened by expert reviewers and grouped into two categories according to their likely relevance after rapid assessment of titles and, when available, abstracts:

1. Obviously not relevant: 485 summary records.
These summary records (titles and/or abstracts) did not contain specific information relevant to the criteria specified in Table 1.

2. Not excluded after rapid assessment: 34 summary records were classified as potentially relevant and thus were assessed in detail, a full assessment of the full-text documents.

3. Following assessment 26 of the full text documents were excluded from the dossier.

4. Following assessment 8 of the full text documents were included in the dossier.

Table 3: Results of the study selection process, for each data requirement or group of data requirements searched

Data requirement(s) captured in the search (as indicated in Table 2)	**n**
Total number of *summary* records retrieved after *all* searches of peer-reviewed literature (excluding duplicates)	519
Number of *summary records* excluded from the search results after rapid assessment for relevance	485
Total number of *full-text documents* assessed in detail	34
Number of *studies* excluded from further consideration after detailed assessment for relevance	26
Number of *studies* included for relevance after detailed assessment	8

Table 4: Report of all relevant studies that are included in a dossier after detailed assessment of full-text documents for relevance: ordered by data requirement(s)

Data requirement (indicated by the corresponding OECD data point number)	**Author(s)**	**Year**	**Title**	**Source**
BIOLOGICAL PROPERTIES OF THE MICRO-ORGANISM				
MMA2.3	Mohan, M. [Reprint Author]; Rangeshwaran, R.; Sivakumar, G.; Verghese, A.	2014	Relative toxicity of subspecies of *Bacillus thuringiensis* against lepidopterous insect pests of agricultural importance.	Journal of Biological Control, (DEC 2014) Vol. 28, No. 4, pp. 197-203. http://journalofbiologicalcontrol.com/. ISSN: 0971-930X. E-ISSN: 0970-5732.
MMA 2.8	de la Vega, Luis Morales; Barboza-Corona, J. Eleazar; Aguilar-Uscanga, Maria G.; Ramirez-Lepe, Mario [Re-print Author]	2006	Purification and characterization of an exo-chitinase from *Bacillus thuringiensis* subsp *aizawai* and its action against phytopathogenic fungi.	Canadian Journal of Microbiology, (JUL 2006) Vol. 52, No. 7, pp. 651-657. CODEN: CJMIAZ. ISSN: 0008-4166. E-ISSN: 1480-3275.
MMA 2.8	Hsieh, Feng-Chia; Lin, Tsung-Chun; Meng, Menghsiao [Reprint Author];	2008	Comparing methods for identifying Bacillus strains capable of producing the antifungal	Current Microbiology, (JAN 2008) Vol. 56, No. 1, pp. 1-5. CODEN:

Data requirement (indicated by the corresponding OECD data point number)	Author(s)	Year	Title	Source
	Kao, Suey-Sheng		lipopeptide iturin A.	CUMIDD. ISSN: 0343-8651.
EFFECTS ON HUMAN HEALTH				
MMA 5.1	Berlitz, Diouneia Lisiane [Reprint Author]; Giovenardi, Marcia; Fiuza, Lidia Mariana	2006	Toxicology effects of delta-endotoxins and beta-exotoxins of *Bacillus thuringiensis* in Wistar rats.	Neotropical Biology and Conservation, (MAY-AUG 2006) Vol. 1, No. 1, pp. 35-38. ISSN: 1809-9939.
MMA 5.3	Lemos, Ana Janaina J. M.; Siqueira, Herbert A. A.; Wanderley-Teixeira, Valeria [Reprint Author]; Maia, Frederico C. L.; Teixeira, Alvaro A. C.; Silva, Edson J.; Oliveira, Jose V.	2013	Effect of sub-lethal doses of *Bacillus thuringiensis* subsp *Aizawai* and deltamethrin with regard to fertility and organ toxicity in pregnant albino rats.	Experimental and Toxicologic Pathology, (JUL 2013) Vol. 65, No. 5, pp. 489-495. CODEN: ETPAEK. ISSN: 0940-2993. E-ISSN: 1618-1433.
MMA 5.3	Lemos, Ana Janaina Jeanine M.; Wanderley-Teixeira, Valeria [Reprint Author]; Teixeira, Alvaro Aguiar C.; Silva, Fernanda das Chagas A.; Oliveira, Jose V.; de Siqueira, Herbert Alvaro A.	2011	Response of blastocyst-endometrium interactions in albino rats to sublethal doses of biological and synthetic insecticides.	Food and Chemical Toxicology, (OCT 2011) Vol. 49, No. 10, pp. 2541-2547. CODEN: FCTOD7. ISSN: 0278-6915. E-ISSN: 1873-6351.
RESIDUES IN OR ON TREATED PRODUCTS, FOOD AND FEED				
MMA 6.1	Frederiksen, Kristine; Rosenquist, Hanne; Jorgensen, Kirsten; Wilcks, Andrea [Reprint Author]	2006	Occurrence of natural *Bacillus thuringiensis* contaminants and residues of *Bacillus thuringiensis*-based insecticides on fresh fruits and vegetables.	Applied and Environmental Microbiology, (MAY 2006) Vol. 72, No. 5, pp. 3435-3440. CODEN: AEMIDF. ISSN: 0099-2240.
EFFECTS ON NON-TARGET ORGANISMS				
MMP 10.3.2	Mommaerts, Veerle; Jans, Kris; Smagghe, Guy [Reprint Author]	2010	Impact of *Bacillus thuringiensis* strains on survival, reproduction and foraging behaviour in bumblebees (Bombus terrestris).	Pest Management Science, (MAY 2010) Vol. 66, No. 5, pp. 520-525. ISSN: 1526-498X.

Table 6: Report of studies excluded from the risk assessment after detailed assessment of full-text documents

Author(s)	Year	Title	Source	Reason(s) for not including this study in the dossier (reliability criteria, point 3 above)
BIOLOGICAL PROPERTIES OF THE MICRO-ORGANISM				
Apaydin, Ozgur; Cinar, Celenk; Turanli, Ferit; Harsa, Sebnem; Gunes, Hatice [Reprint Author]	2008	Identification and bioactivity of native strains of *Bacillus thuringiensis* from grain-related habitats in Turkey.	Biological Control, (APR 2008) Vol. 45, No. 1, pp. 21-28. ISSN: 1049-9644.	This paper appears to relate to cereal grain which is not relevant for the uses proposed for this submission (brassicas, tomatoes and peppers). The paper would not therefore be considered by the authorities in support of this submission.
Basedow, T.; El-Shafie, H. A. F.; Abo-El-Saad, M. M.; Al-Ajlan, A. M.	2012	Evaluation of *Bacillus thuringiensis aizawai* and neem for controlling the larvae of the Greater Wax Moth, Galleria mellonella (Lepidoptera: Pyralidae).	International Journal of Agriculture and Biology (2012), Volume 14, Number 4, pp. 629-632, 21 refs. ISSN: 1560-8530 Published by: Friends Science Publishers, Faisalabad URL (Availability): http://www.fspublishers.org/ijab/past-issues/IJABVOL_14_NO_4/27.pdf	Not determined to be relevant
Hatem, A. E.	2014	Lethal and sublethal effects of spinosad and Bacillus thuringiensis aizawai on reproductivity of Spodoptera littroralis (boisd.) and Spodoptera exigua (huebner) (Lepidoptera: Noctuidae).	Egyptian Journal of Biological Pest Control (2014), Volume 24, Number 1, pp. 65-69, 22 refs. ISSN: 1110-1768 Published by: Egyptian Society for Biological Control of Pests, Cairo URL (Availability): http://www.esbcp.org/index.asp	Not determined to be relevant
Maxwell, Elly M. [Reprint Author]; Fadamiro, Henry Y.	2006	Evaluation of several reduced-risk insecticides in combination with an action threshold for managing lepidopteran pests of cole crops in Alabama.	Florida Entomologist, (JUN 2006) Vol. 89, No. 2, pp. 117-126. CODEN: FETMAC. ISSN: 0015-4040.	Not determined to be relevant
Pandey, Shachindra [Reprint Author]; Joshi, Bishwambhar D.; Tiwari, Lakshmi D.	2009	Relative efficacy of two subspecies of Bacillus thuringiensis, available as commercial preparations in market, on different stages of a lepidopteran pest, Spodoptera litura (Fabricius).	Archives of Phytopathology and Plant Protection, (2009) Vol. 42, No. 10, pp. 903-914. http://www.tandfonline.com/loi/gapp20. CODEN: APPZAJ. ISSN: 0323-5408. E-ISSN: 1477-2906.	Not determined to be relevant
Pedroso de Moraes, Carla; Foerster, Luis Amilton	2012	Toxicity and residual control of Plutella xylostella L. (Lepidoptera: Plutellidae) with Bacillus thuringiensis Berliner and insecticides	Ciencia Rural (2012), 42(8), 1335-1340 CODEN: CIRUEP; ISSN: 0103-8478	Not determined to be relevant
Silva, Maria C.; Siqueira,	2012	Bacillus thuringiensis isolates from	Biocontrol Science and Technology, (2012) Vol. 22, No. 5,	Not determined to be relevant

Author(s)	Year	Title	Source	Reason(s) for not including this study in the dossier (reliability criteria, point 3 above)
Herbert A. A. [Reprint Author]; Marques, Edmilson J.; Silva, Liliane M.; Barros, Reginaldo; Lima Filho, Jose V. M.; Silva, Suzana M. F. A.		northeastern Brazil and their activities against Plutella xylostella (Lepidoptera: Plutellidae) and Spodoptera frugiperda (Lepidoptera: Noctuidae).	pp. 583-599. http://www.tandfonline.com/loi/cbst20. ISSN: 0958-3157. E-ISSN: 1360-0478.	
Stein, H. H.; Sauber, T. E.; Rice, D. W.; Hinds, M. A.; Smith, B. L.; Dana, G.; Peters, D. N.; Hunst, P.	2009	Growth performance and carcass composition of pigs fed corn grain from das-o15o7-1 (herculex i) hybrids 1.	Professional Animal Scientist (2009), Volume 25, Number 6, pp. 689-694 ISSN: 1080-7446 Published by: American Registry of Professional Animal Scientists (ARPAS), Illinois URL (Availability): http://pas.fass.org/content/25/6/689.abstract	Not determined to be relevant
Yan, S.; Mohammedi, S.; Tyagi, R. D.; Surampalli, R. Y.; Valero, J. R.	2007	Growth of Four Serovar of Bacillus thuringiensis (Var. Kurstaki, Israelensis, Tenebrionis, and Aizawai) in Wastewater Sludge	Practice Periodical of Hazardous, Toxic, and Radioactive Waste Management (2007), 11(2), 123-129 CODEN: PPHMF8; ISSN: 1090-025X	Study on the levels of Bt on sludge and not relevant for the residues or analytical methods sections
EFFECTS ON HUMAN HEALTH				
Apaydin, Ozgur; Cinar, Celenk; Turanli, Ferit; Harsa, Sebnem; Gunes, Hatice [Reprint Author]	2008	Identification and bioactivity of native strains of *Bacillus thuringiensis* from grain-related habitats in Turkey.	Biological Control, (APR 2008) Vol. 45, No. 1, pp. 21-28. ISSN: 1049-9644.	This paper appears to relate to cereal grain which is not relevant for the uses proposed for this submission (brassicas, tomatoes and peppers). The paper would not therefore be considered by the authorities in support of this submission.
BenFarhat-Touzri, Dalel; Saadaoui, Marwa; Abdelkefi-Mesrati, Lobna; Saadaoui, Imen; Azzouz, Hichem; Tounsi, Slim [Reprint Author]	2013	Histopathological effects and determination of the putative receptor of *Bacillus thuringiensis* Cry1Da toxin in Spodoptera littoralis midgut.	Journal of Invertebrate Pathology, (FEB 2013) Vol. 112, No. 2, pp. 142-145. http://www.journals.elsevier.com/journal-of-invertebrate-pathology/#description. CODEN: JIVPAZ. ISSN: 0022-2011. E-ISSN: 1096-0805.	Not determined to be relevant
El-Aziz, S. H. A.; El-Gohary, E. E.; Mansy, M. S.; Desuky, W. M.;	2012	Toxicological and biochemical studies on development of resistance in Spodoptera littoralis (Boisd.) during	The Journal of American Science (2012), Volume 8, Number 1, pp. 418-426, 39 refs. ISSN: 1545-1003 Published by: Marsland Press, New York URL (Availability):	Not determined to be relevant

Author(s)	Year	Title	Source	Reason(s) for not including this study in the dossier (reliability criteria, point 3 above)
Hamed, M. S.		selection with Bacillus thuringiensis MVPII.	http://www.jofamericanscience.org/journals/am-sci/am0801/059_7571am0801_418_426.pdf	
Hommes, M.	2010	Investigations on integrated pest management in white cabbage. Untersuchungen zur integrierten Bekaempfung von Schaedlingen an Weisskohl.	Julius-Kuehn-Archiv (2010), Number 428, pp. 215-216 ISSN: 1868-9892 Published by: Julius Kuehn Institut, Bundesforschungsinstitut fuer Kulturpflanzen, Quedlinburg Conference: 57. Deutsche Pflanzenschutztagung, Berlin, Germany, 6-9 September, 2010. URL (Availability): http://pub.jki.bund.de/index.php/JKA/issue/archive	Not determined to be relevant
Kim, Min-Ju; Han, Jae-Kwang; Park, Jong-Su; Lee, Jin-Sung; Lee, Soon-Ho; Cho, Joon-Il; Kim, Keun-Sung [Reprint Author]	2015	Various Enterotoxin and Other Virulence Factor Genes Widespread Among Bacillus cereus and *Bacillus thuringiensis* Strains.	Journal of Microbiology and Biotechnology, (JUN 2015) Vol. 25, No. 6, pp. 872-879. http://jmb.or.kr. ISSN: 1017-7825. E-ISSN: 1738-8872.	Not determined to be relevant
Kumar, Prabhat [Reprint Author]; Huang, Lu-Ying Zoe; Srinivasan, R.	2014	Effect of three commercial biopesticides of neem (Azadirachta indica) and *Bacillus thuringiensis* on legume pod borer (Maruca vitrata) (Lepidoptera: Crambidae) in Thailand.	International Journal of Tropical Insect Science, (JUN 2014) Vol. 34, No. 2, pp. 80-87. http://journals.cambridge.org/action/displayJournal?jid=JTI. ISSN: 1742-7584. E-ISSN: 1742-7592.	Not determined to be relevant
MacKenzie, Susan A.; Lamb, Ian; Schmidt, Jean; Deege, Lora; Morrisey, Michael J.; Harper, Marc; Layton, Raymond J.; Prochaska, Lee M.; Sanders, Craig; Locke, Mary; Mattsson, Joel L.; Fuentes, Angel; Delaney, Bryan [Reprint Author]	2007	Thirteen week feeding study with transgenic maize grain containing event DAS-O15O7-1 in Sprague-Dawley rats.	Food and Chemical Toxicology, (APR 2007) Vol. 45, No. 4, pp. 551-562. CODEN: FCTOD7. ISSN: 0278-6915.	Not determined to be relevant
Mashtoly, Tamer A.; Abolmaaty, Assem; El-Zemaity, Mohamed El-Said; Hussien, Mohamed I.; Alm, Steven R. [Re-	2011	Enhanced Toxicity of *Bacillus thuringiensis* Subspecies kurstaki and *aizawai* to Black Cutworm Larvae (Lepidoptera: Noctuidae) With Bacillus sp NFD2 and Pseudomonas sp FNFD1.	Journal of Economic Entomology, (FEB 2011) Vol. 104, No. 1, pp. 41-46. CODEN: JEENAI. ISSN: 0022-0493. E-ISSN: 1938-291X.	Not determined to be relevant

Author(s)	Year	Title	Source	Reason(s) for not including this study in the dossier (reliability criteria, point 3 above)
print Author]				
Mommaerts, V.; Sterk, G.; Smagghe, G. Editor(s): Oomen, P. A.; Thompson, H. M.	2009	Side effects of commercial *Bacillus thuringiensis* insecticides on micro-colonies of Bombus terrestris.	Julius-Kuehn-Archiv (2009), Number 423, pp. 68-69 ISSN: 1868-9892 Published by: Julius Kuehn Institut, Bundesforschungsinstitut fuer Kulturpflanzen, Quedlinburg Conference: Hazards of pesticides to bees. 10th International Symposium of the ICP-Bee Protection Group. Bucharest, Romania, 8-10 October, 2008. URL (Availability): http://pub.jki.bund.de/index.php/JKA/issue/archive	Conference abstract only. Full study details are available in the published paper referenced also in the literature search.
Pedroso de Moraes, Carla; Foerster, Luis Amilton	2012	Toxicity and residual control of Plutella xylostella L. (Lepidoptera: Plutellidae) with *Bacillus thuringiensis* Berliner and insecticides	Ciencia Rural (2012), 42(8), 1335-1340 CODEN: CIRUEP; ISSN: 0103-8478	Not determined to be relevant
Ratanasatien, F.; Ketunuti, U.; Tantichodok, A.; Faisan Ratanasatien; Uthai Ketunuti; Achara Tantichodok Editor(s): Cote, J. C.; Otvos, I. S.; Schwartz, J. L.; Vincent, C.	2007	Positioning of biopesticides in Thailand.	Proceedings of the 6th Pacific Rim Conference on the biotechnology of *Bacillus thuringiensis* and its environmental impact, Victoria, BC, Canada, 30 October - 3 November, 2005 (2007), pp. 100-107, 16 refs. ISBN: 978-2-9810223-0-1 Published by: National Sciences and Engineering Research Council of Canada (NSERC), Ottawa Conference: Proceedings of the 6th Pacific Rim Conference on the biotechnology of *Bacillus thuringiensis* and its environmental impact, Victoria, BC, Canada, 30 October - 3 November, 2005.	Not determined to be relevant
Silva, Maria C.; Siqueira, Herbert A. A. [Reprint Author]; Marques, Edmilson J.; Silva, Liliane M.; Barros, Reginaldo; Lima Filho, Jose V. M.; Silva, Suzana M. F. A.	2012	*Bacillus thuringiensis* isolates from northeastern Brazil and their activities against Plutella xylostella (Lepidoptera: Plutellidae) and Spodoptera frugiperda (Lepidoptera: Noctuidae).	Biocontrol Science and Technology, (2012) Vol. 22, No. 5, pp. 583-599. http://www.tandfonline.com/loi/cbst20. ISSN: 0958-3157. E-ISSN: 1360-0478.	Not determined to be relevant
Vicidomini, S.; Bernardo, U.; Laudonia, S.; Sannino, L.	2006	Lethal and sublethal effects of *Bacillus thuringiensis* Berliner ssp. *aizawai* on Spodoptera littoralis (Boisduval) (Lepidoptera: Noctuidae) larvae in extended laboratory tests. Effetti letali e subletali di *Bacillus thuringiensis* Berliner ssp.	Bollettino del Laboratorio di Entomologia Agraria "Filippo Silvestri" (2006), Volume 61, pp. 53-61, 32 refs. ISSN: 0304-0658 Published by: Dipartimento di Entomologia e Zoologia Agriaria dell'Universita di Napoli Frederico II, Portici	Not determined to be relevant

Author(s)	Year	Title	Source	Reason(s) for not including this study in the dossier (reliability criteria, point 3 above)
		aizawai su larve di Spodoptera littoralis (Boisduval) (Lepidoptera: Noctuidae) in prove di laboratorio esteso.		
Wanapaisan, P.; Chumsakul, O.; Panbangred, W. [Reprint Author]	2013	Enhanced Cry1Da production in *Bacillus thuringiensis* by driving expression from the sigma(E)-dependent BtI promoter.	Journal of Applied Microbiology, (SEP 2013) Vol. 115, No. 3, pp. 859-871. http://onlinelibrary.wiley.com/journal/10.1111/(ISSN)1365-2672. ISSN: 1364-5072. E-ISSN: 1365-2672.	Not determined to be relevant
Yan, S.; Mohammedi, S.; Tyagi, R. D.; Surampalli, R. Y.; Valero, J. R.	2007	Growth of Four Serovar of *Bacillus thuringiensis* (Var. Kurstaki, Israelensis, Tenebrionis, and *Aizawai*) in Wastewater Sludge	Practice Periodical of Hazardous, Toxic, and Radioactive Waste Management (2007), 11(2), 123-129 CODEN: PPHMF8; ISSN: 1090-025X	Study on the levels of Bt on sludge and not relevant for the residues or analytical methods sections
Zhou, Guoping; Yan, Jianping; Zheng Dasheng; Zhou, Xiaohui; Yuan, Zhiming [Reprint Author]	2008	The residual occurrences of *Bacillus thuringiensis* biopesticides in food and beverages.	International Journal of Food Microbiology, (SEP 30 2008) Vol. 127, No. 1-2, pp. 68-72. http://www.journals.elsevier.com/international-journal-of-food-microbiology/#description. CODEN: IJFMDD. ISSN: 0168-1605. E-ISSN: 1879-3460.	Focus of paper is levels in tea and dairy products. Based on China-authorised B. thuringiensis products. Paper does not describe which strains are commerically available in China therefore the information is not specific.
RESIDUES IN OR ON TREATED PRODUCTS, FOOD AND FEED				
Ratanasatien, F.; Ketunuti, U.; Tantichodok, A.; Faisan Ratanasatien; Uthai Ketunuti; Achara Tantichodok Editor(s): Cote, J. C.; Otvos, I. S.; Schwartz, J. L.; Vincent, C.	2007	Positioning of biopesticides in Thailand.	Proceedings of the 6th Pacific Rim Conference on the biotechnology of *Bacillus thuringiensis* and its environmental impact, Victoria, BC, Canada, 30 October - 3 November, 2005 (2007), pp. 100-107, 16 refs. ISBN: 978-2-9810223-0-1 Published by: National Sciences and Engineering Research Council of Canada (NSERC), Ottawa Conference: Proceedings of the 6th Pacific Rim Conference on the biotechnology of *Bacillus thuringiensis* and its environmental impact, Victoria, BC, Canada, 30 October - 3 November, 2005.	Not determined to be relevant
Stephan, D.; Scholz-Doebelin, H.; Reintges, T.; Pelz, J.; Jehle, J. A.; Kessler, J.	2014	Investigations on residues of XenTari®(*Bacillus thuringiensis* subspec. *aizawai*) on greenhouse tomatoes.	Journal fuer Kulturpflanzen (2014), Volume 66, Number 9, pp. 312-318, 21 refs. ISSN: 1867-0911 Published by: Eugen Ulmer KG, Stuttgart URL (Availability): http://www.journal-kulturpflanzen.de	Paper not relevant because the study was not conducted at a GAP sufficient to support the critical GAP proposed for this

Author(s)	Year	Title	Source	Reason(s) for not including this study in the dossier (reliability criteria, point 3 above)
				submission.
	2013	Conclusion on the peer review of the pesticide risk assessment of the active substance *Bacillus thuringiensis* subsp. *aizawai* (strains ABTS 1857, GC-91).	EFSA Journal (2013), Volume 11, Number 1, 3063 p., 11 refs. ISSN: 1831-4732 Published by: European Food Safety Authority, Parma URL (Availability): http://www.efsa.europa.eu/en/efsajournal/doc/3063.pdf	Not determined to be relevant
FATE AND BEHAVIOUR IN THE ENVIRONMENT				
Basedow, T.; El-Shafie, H. A. F.; Abo-El-Saad, M. M.; Al-Ajlan, A. M.	2012	Evaluation of *Bacillus thuringiensis aizawai* and neem for controlling the larvae of the Greater Wax Moth, Galleria mellonella (Lepidoptera: Pyralidae).	International Journal of Agriculture and Biology (2012), Volume 14, Number 4, pp. 629-632, 21 refs. ISSN: 1560-8530 Published by: Friends Science Publishers, Faisalabad URL (Availability): http://www.fspublishers.org/ijab/past-issues/IJABVOL_14_NO_4/27.pdf	Not determined to be relevant
Mommaerts, V.; Sterk, G.; Smagghe, G. Editor(s): Oomen, P. A.; Thompson, H. M.	2009	Side effects of commercial *Bacillus thuringiensis* insecticides on microcolonies of Bombus terrestris.	Julius-Kuehn-Archiv (2009), Number 423, pp. 68-69 ISSN: 1868-9892 Published by: Julius Kuehn Institut, Bundesforschungsinstitut fuer Kulturpflanzen, Quedlinburg Conference: Hazards of pesticides to bees. 10th International Symposium of the ICP-Bee Protection Group. Bucharest, Romania, 8-10 October, 2008. URL (Availability): http://pub.jki.bund.de/index.php/JKA/issue/archive	Conference abstract only. Full study details are available in the published paper referenced also in the literature search.
Silva, Maria C.; Siqueira, Herbert A. A. [Reprint Author]; Marques, Edmilson J.; Silva, Liliane M.; Barros, Reginaldo; Lima Filho, Jose V. M.; Silva, Suzana M. F. A.	2012	*Bacillus thuringiensis* isolates from northeastern Brazil and their activities against Plutella xylostella (Lepidoptera: Plutellidae) and Spodoptera frugiperda (Lepidoptera: Noctuidae).	Biocontrol Science and Technology, (2012) Vol. 22, No. 5, pp. 583-599. http://www.tandfonline.com/loi/cbst20. ISSN: 0958-3157. E-ISSN: 1360-0478.	Not determined to be relevant
EFFECTS ON NON-TARGET ORGANISMS				
Aggarwal, N.; Holaschke, M.; Basedow, T.	2006	Evaluation of bio-rational insecticides to control Helicoverpa armigera (Huebner) and Spodoptera exigua (Huebner) (Lepidoptera: Noctuidae) fed on Vicia faba L.	Mitteilungen der Deutschen Gesellschaft fuer allgemeine und angewandte Entomologie (2006), Volume 15, pp. 245-250, 16 refs. ISSN: 0344-9084 Published by: Deutsche Gesellschaft fuer allgemeine und angewandte Entomologie e.V., Bayreuth Conference: Papers from the Entomological Conference in Dresden, Germany, 21-24 March 2005.	Not determined to be relevant

Author(s)	Year	Title	Source	Reason(s) for not including this study in the dossier (reliability criteria, point 3 above)
Apaydin, Ozgur; Cinar, Celenk; Turanli, Ferit; Harsa, Sebnem; Gunes, Hatice [Reprint Author]	2008	Identification and bioactivity of native strains of Bacillus thuringiensis from grain-related habitats in Turkey.	Biological Control, (APR 2008) Vol. 45, No. 1, pp. 21-28. ISSN: 1049-9644.	This paper appears to relate to cereal grain which is not relevant for the uses proposed for this submission (brassicas, tomatoes and peppers). The paper would not therefore be considered by the authorities in support of this submission.
Basedow, T.; Ahmad, M.; Tadesse, B.; El-Shafie, H.	2008	Galleria mellonella (L.) (Pyralidae) and Spodoptera exigua (Huebner) (Noctuidae): differences in effects of XenTari® (Bacillus thuringiensis aizawai), NeemAzal T/S® and their combinations on survival.	Mitteilungen der Deutschen Gesellschaft fuer allgemeine und angewandte Entomologie (2008), Volume 16, pp. 365-368, 24 refs. ISSN: 0344-9084 Published by: Deutsche Gesellschaft fuer allgemeine und angewandte Entomologie e.V., MXFFFDncheberg Conference: Vortraege der Entomologentagung, Innsbruck, Austria, 26 February to 1 March, 2007. URL (Availability): http://www.dgaae.de	Not determined to be relevant
Basedow, T.; El-Shafie, H. A. F.; Abo-El-Saad, M. M.; Al-Ajlan, A. M.	2012	Evaluation of *Bacillus thuringiensis aizawai* and neem for controlling the larvae of the Greater Wax Moth, Galleria mellonella (Lepidoptera: Pyralidae).	International Journal of Agriculture and Biology (2012), Volume 14, Number 4, pp. 629-632, 21 refs. ISSN: 1560-8530 Published by: Friends Science Publishers, Faisalabad URL (Availability): http://www.fspublishers.org/ijab/past-issues/IJABVOL_14_NO_4/27.pdf	Not determined to be relevant
BenFarhat-Touzri, Dalel; Saadaoui, Marwa; Abdelkefi-Mesrati, Lobna; Saadaoui, Imen; Azzouz, Hichem; Tounsi, Slim [Reprint Author]	2013	Histopathological effects and determination of the putative receptor of *Bacillus thuringiensis* Cry1Da toxin in Spodoptera littoralis midgut.	Journal of Invertebrate Pathology, (FEB 2013) Vol. 112, No. 2, pp. 142-145. http://www.journals.elsevier.com/journal-of-invertebrate-pathology/#description. CODEN: JIVPAZ. ISSN: 0022-2011. E-ISSN: 1096-0805.	Not determined to be relevant
El-Aziz, S. H. A.; El-Gohary, E. E.; Mansy, M. S.; Desuky, W. M.; Hamed, M. S.	2012	Toxicological and biochemical studies on development of resistance in Spodoptera littoralis (Boisd.) during selection with *Bacillus thuringiensis* MVPII.	The Journal of American Science (2012), Volume 8, Number 1, pp. 418-426, 39 refs. ISSN: 1545-1003 Published by: Marsland Press, New York URL (Availability): http://www.jofamericanscience.org/journals/am-sci/am0801/059_7571am0801_418_426.pdf	Not determined to be relevant
Gonzalez-Cabrera, J.; Molla, O.; Urbaneja, A.	2009	Biological control of Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) with *Bacillus thuringiensis* (Berliner).	Agricola Vergel: Fruticultura, Horticultura, Floricultura, Citricultura, Vid, Arroz (2009), Volume 28, Number 333, pp. 476-480, 17 refs. ISSN: 0211-2728 Published by: Edi-	Not determined to be relevant

Author(s)	Year	Title	Source	Reason(s) for not including this study in the dossier (reliability criteria, point 3 above)
		Control biologico de Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) con *Bacillus thuringiensis* (Berliner).	ciones y Promociones L.A.V., Valencia URL (Availability): http://www.edicioneslav.com	
Hatem, A. E.	2014	Lethal and sublethal effects of spinosad and Bacillus thuringiensis aizawai on reproductivity of Spodoptera littroralis (boisd.) and Spodoptera exigua (huebner) (Lepidoptera: Noctuidae).	Egyptian Journal of Biological Pest Control (2014), Volume 24, Number 1, pp. 65-69, 22 refs. ISSN: 1110-1768 Published by: Egyptian Society for Biological Control of Pests, Cairo URL (Availability): http://www.esbcp.org/index.asp	Not determined to be relevant
Hommes, M.	2010	Investigations on integrated pest management in white cabbage. Untersuchungen zur integrierten Bekaempfung von Schaedlingen an Weisskohl.	Julius-Kuehn-Archiv (2010), Number 428, pp. 215-216 ISSN: 1868-9892 Published by: Julius Kuehn Institut, Bundesforschungsinstitut fuer Kulturpflanzen, Quedlinburg Conference: 57. Deutsche Pflanzenschutztagung, Berlin, Germany, 6-9 September, 2010. URL (Availability): http://pub.jki.bund.de/index.php/JKA/issue/archive	Not determined to be relevant
Kim, Min-Ju; Han, Jae-Kwang; Park, Jong-Su; Lee, Jin-Sung; Lee, Soon-Ho; Cho, Joon-Il; Kim, Keun-Sung [Reprint Author]	2015	Various Enterotoxin and Other Virulence Factor Genes Widespread Among Bacillus cereus and Bacillus thuringiensis Strains.	Journal of Microbiology and Biotechnology, (JUN 2015) Vol. 25, No. 6, pp. 872-879. http://jmb.or.kr. ISSN: 1017-7825. E-ISSN: 1738-8872.	Not determined to be relevant
Kumar, Prabhat [Reprint Author]; Huang, Lu-Ying Zoe; Srinivasan, R.	2014	Effect of three commercial biopesticides of neem (Azadirachta indica) and *Bacillus thuringiensis* on legume pod borer (Maruca vitrata) (Lepidoptera: Crambidae) in Thailand.	International Journal of Tropical Insect Science, (JUN 2014) Vol. 34, No. 2, pp. 80-87. http://journals.cambridge.org/action/displayJournal?jid=JTI. ISSN: 1742-7584. E-ISSN: 1742-7592.	Not determined to be relevant
Mashtoly, Tamer A.; Abolmaaty, Assem; El-Zemaity, Mohamed El-Said; Hussien, Mohamed I.; Alm, Steven R. [Reprint Author]	2011	Enhanced Toxicity of *Bacillus thuringiensis* Subspecies kurstaki and *aizawai* to Black Cutworm Larvae (Lepidoptera: Noctuidae) With Bacillus sp NFD2 and Pseudomonas sp FNFD1.	Journal of Economic Entomology, (FEB 2011) Vol. 104, No. 1, pp. 41-46. CODEN: JEENAI. ISSN: 0022-0493. E-ISSN: 1938-291X.	Not determined to be relevant
Maxwell, Elly M. [Reprint Author]; Fadamiro, Henry Y.	2006	Evaluation of several reduced-risk insecticides in combination with an action threshold for managing lepidopteran	Florida Entomologist, (JUN 2006) Vol. 89, No. 2, pp. 117-126. CODEN: FETMAC. ISSN: 0015-4040.	Not determined to be relevant

Author(s)	Year	Title	Source	Reason(s) for not including this study in the dossier (reliability criteria, point 3 above)
		pests of cole crops in Alabama.		
Mommaerts, V.; Sterk, G.; Smagghe, G. Editor(s): Oomen, P. A.; Thompson, H. M.	2009	Side effects of commercial *Bacillus thuringiensis* insecticides on micro-colonies of Bombus terrestris.	Julius-Kuehn-Archiv (2009), Number 423, pp. 68-69 ISSN: 1868-9892 Published by: Julius Kuehn Institut, Bundesforschungsinstitut fuer Kulturpflanzen, Quedlinburg Conference: Hazards of pesticides to bees. 10th International Symposium of the ICP-Bee Protection Group. Bucharest, Romania, 8-10 October, 2008. URL (Availability): http://pub.jki.bund.de/index.php/JKA/issue/archive	Conference abstract only. Full study details are available in the published paper referenced also in the literature search.
Pandey, Shachindra [Reprint Author]; Joshi, Bishwambhar D.; Tiwari, Lakshmi D.	2009	Relative efficacy of two subspecies of Bacillus thuringiensis, available as commercial preparations in market, on different stages of a lepidopteran pest, Spodoptera litura (Fabricius).	Archives of Phytopathology and Plant Protection, (2009) Vol. 42, No. 10, pp. 903-914. http://www.tandfonline.com/loi/gapp20. CODEN: APPZAJ. ISSN: 0323-5408. E-ISSN: 1477-2906.	Not determined to be relevant
Pedroso de Moraes, Carla; Foerster, Luis Amilton	2012	Toxicity and residual control of Plutella xylostella L. (Lepidoptera: Plutellidae) with Bacillus thuringiensis Berliner and insecticides	Ciencia Rural (2012), 42(8), 1335-1340 CODEN: CIRUEP; ISSN: 0103-8478	Not determined to be relevant
Ratanasatien, F.; Ketunuti, U.; Tantichodok, A.; Faisan Ratanasatien; Uthai Ketunuti; Achara Tantichodok Editor(s): Cote, J. C.; Otvos, I. S.; Schwartz, J. L.; Vincent, C.	2007	Positioning of biopesticides in Thailand.	Proceedings of the 6th Pacific Rim Conference on the biotechnology of Bacillus thuringiensis and its environmental impact, Victoria, BC, Canada, 30 October - 3 November, 2005 (2007), pp. 100-107, 16 refs. ISBN: 978-2-9810223-0-1 Published by: National Sciences and Engineering Research Council of Canada (NSERC), Ottawa Conference: Proceedings of the 6th Pacific Rim Conference on the biotechnology of Bacillus thuringiensis and its environmental impact, Victoria, BC, Canada, 30 October - 3 November, 2005.	Not determined to be relevant
Silva, Maria C.; Siqueira, Herbert A. A. [Reprint Author]; Marques, Edmilson J.; Silva, Liliane M.; Barros, Reginaldo; Lima Filho, Jose V. M.; Silva, Suzana M. F. A.	2012	*Bacillus thuringiensis* isolates from northeastern Brazil and their activities against Plutella xylostella (Lepidoptera: Plutellidae) and Spodoptera frugiperda (Lepidoptera: Noctuidae).	Biocontrol Science and Technology, (2012) Vol. 22, No. 5, pp. 583-599. http://www.tandfonline.com/loi/cbst20. ISSN: 0958-3157. E-ISSN: 1360-0478.	Not determined to be relevant
Vicidomini, S.; Bernardo,	2006	Lethal and sublethal effects of *Bacillus*	Bollettino del Laboratorio di Entomologia Agraria "Filippo	Not determined to be relevant

Author(s)	Year	Title	Source	Reason(s) for not including this study in the dossier (reliability criteria, point 3 above)
U.; Laudonia, S.; Sannino, L.		*thuringiensis* Berliner ssp. *aizawai* on Spodoptera littoralis (Boisduval) (Lepidoptera: Noctuidae) larvae in extended laboratory tests. Effetti letali e subletali di *Bacillus thuringiensis* Berliner ssp. *aizawai* su larve di Spodoptera littoralis (Boisduval) (Lepidoptera: Noctuidae) in prove di laboratorio esteso.	Silvestri" (2006), Volume 61, pp. 53-61, 32 refs. ISSN: 0304-0658 Published by: Dipartimento di Entomologia e Zoologia Agriaria dell'Universita di Napoli Frederico II, Portici	
Wanapaisan, P.; Chumsakul, O.; Panbangred, W. [Reprint Author]	2013	Enhanced Cry1Da production in *Bacillus thuringiensis* by driving expression from the sigma(E)-dependent BtI promoter.	Journal of Applied Microbiology, (SEP 2013) Vol. 115, No. 3, pp. 859-871. http://onlinelibrary.wiley.com/journal/10.1111/(ISSN)1365-2672. ISSN: 1364-5072. E-ISSN: 1365-2672.	Not determined to be relevant

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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
MMA 5.1/01	Shimada N, Miyamoto K, Kanda K & Murata H	2006	*Bacillus thuringiensis* insecticidal Cry1Ab toxin does not affect the membrane integrity of the mammalian intestinal epithelial cells: An *in vitro* study. In Vitro Cellular & Developmental Biology Animal 42 (1-2):45-49. GLP: no Published	N	N	-	-

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
MMA 5.1/02	Berlitz DL	2006	Toxicology effects of δ-endotoxins and β-exotoxins of *Bacillus thuringiensis* in Wistar rats. Neotropical Biology and Conservation 1(1):35-38 GLP: no Published	Y	N	-	-
MMA 5.1/03	Wilcks A, Hansen BM, Hendricksen NB & Licht TR	2006	Persistence of *Bacillus thuringiensis* bioinsecticides in the gut of human-flora-associated rats. FEMS Immunology & Medical Microbiology 48:410-418. GLP: no Published	Y	N	-	-
MMA 5.1/04	van der Wiele *et al*	2015	Evaluation of the germination behavior of DiPel and XenTari during passage through the GIT and their impact on the gut microbiota. *In vitro* study of immune effects mediated by the fermentation-derived metabolites using Caco-2 / THP1 co-cultures	N	N	New data submitted for the first time	VBC
MMA 5.1/05	Anon	2018	Evaluation of the effect of Bacillus thuringiensis XenTari on Caco-2 cells (follow up Deal 312)	N	Y	New data submitted for the first time	VBC
MMA 5.1.1/01	Glyn S	2016	XenTari® - Bta Untitled Report GLP: no Unpublished	N	N	-	VBC

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**
MMA 5.1.3/01	Baelum J, Larsen P, Doekes G & Sisgaard T	2012	Health effects of selected microbiological control agents: a 3-year follow-up study. Annals of Agricultural and Environmental Medicine 19(4):631-636 GLP: no Published	N	N	-	-
MMA 5.1.4/01	McIntyre L, Bernard K, Beniac D, Isaac-Renton JL & Naseby DC	2008	Identification of *Bacillus cereus* Group Species Associated with Food Poisoning Outbreaks in British Columbia, Canada. Applied & Environmental Microbiology 74(23):7451-7453 GLP: no Published	N	N	-	-
MMA 5.1.4/02	Forrester MB	2012	*Bacillus thuringiensis* pesticide exposures reported to Texas poison centers. Toxicological & Environmental Chemistry 94(4):799-804 GLP: no Published	N	N	-	-

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 protec-tion claimed Y/N	Justification if data protection is claimed	Owner
IIM 5.3.1/01	[redacted]	1997a	XenTari Technical Powder (ABG-6305): Dermal sensitization study in Guinea pig Report number 3088-96 GLP Unpublished (Previously considered in the DAR)	Y	N	-	VBC
IIM 5.3.2/01	[redacted]	1995	Acute oral toxicity/pathogenicity study of *Bacillus thuringiensis* ABG-6305 in rats Report number M94AG90.222.008 GLP Unpublished (Previously considered in the DAR)	Y	N	-	VBC
IIM 5.3.2/02	[redacted]	1996a	XenTari Technical Powder (ABG-6305): Acute oral toxicity study in rats Report number 3084-96 GLP Unpublished (Previously considered in the DAR)	Y	N	-	VBC

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
IIM 5.3.3/02	[redacted]	1999	XenTari Technical Powder (ABG-6305): Acute inhalation toxicity study in rats Report number 4432-98 GLP Unpublished (Previously considered in the DAR)	Y	N	-	VBC
IIM 5.3.4/02	[redacted]	1991a	Acute pulmonary toxicity and infectivity/ pathogenicity to rats of *Bacillus thuringiensis* ABG-6305 Report number 901292D/ABT 143-2/AC GLP Unpublished (Previously considered in the DAR)	Y	N	-	VBC
IIM 5.3.4/01	[redacted]	1990	Intraperitoneal and subcutaneous injection tests with ABG-6305 Technical Powder Report number 85K-11/9 GLP Unpublished (Previously considered in the DAR)	Y	N	-	VBC

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 protec-tion claimed Y/N	Justification if data protection is claimed	Owner
IIM 5.3.4/02	[redacted]	1991b	Acute intravenous toxicity and infectivity/ patho-genicity to rats of ABG-6305 Report number 901291D/ABT 143-1/AC GLP Unpublished (Previously considered in the DAR)	Y	N	-	VBC
IIM 5.3.5/01	Lawlor T	1997	Mutagenicity test with XenTari Technical Powder (ABG-6305) in the Salmonella – Escherichia coli/mammalian- microsome reverse mutation assay Report number 18447-0-409 GLP Unpublished (Previously considered in the DAR)	N	N	-	VBC
MMA 5.2.5.1/01	Hammond BG, Dudek R, Lemen JK & Nemeth MA	2006	Results of a 90-day safety assurance study with rats fed grain from corn borer-protected corn. Food and Chemical Toxicology 44: 1092-1099. GLP: no Published	Y	N	-	-

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**
MMA 5.2.5.1/02	Onose J, Imai T, Hasumura M, Ueda M, Ozeki Y & Hirose M	2008	Evaluation of subchronic toxicity of dietary administered Cry1Ab protein from *Bacillus thuringiensis* var. *Kurustaki* HD-1 in F344 male rats with chemically induced gastrointestinal impairment. Food and Chemical Toxicology 46: 2184–2189. GLP: no Published	Y	N	-	-
MMA 5.3/01	Lemos AJJM, Wanderley-Teixera V, Teixera AAC, Silva FdCA, Oliveira JV , & Siquiera HAA	2011	Response of blastocyst–endometrium interactions in albino rats to sublethal doses of biological and synthetic insecticides. Food & Chemical Pathology 49:2541-2547 GLP: no Published	Y	N	-	-
MMA 5.3/02	Lemos AJJM, Siquiera HAA, Wanderley-Teixera V, Maia FCL, Teixera AAC, Silva EJ & Oliveira JV	2013	Effect of sub-lethal doses of *Bacillus thuringiensis* subsp. *aizawai* and deltamethrin with regard to fertility and organ toxicity in pregnant albino rats GLP: no Published	Y	N	-	-

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**
MMA 5.4/01	Freire IS, Miranda-Vilela AL, Barbosa LCP, Martins ES, Monnerat RG & Grisolia CK	2014a	Evaluation of Cytotoxicity, Genotoxicity and Hematotoxicity of the Recombinant Spore-Crystal Complexes Cry1Ia, Cry10Aa and Cry1Ba6 from *Bacillus thuringiensis* in Swiss Mice. Toxins 6: 2872-2885 GLP: no Published	Y	N	-	-
MMA 5.4/02	Freire IS, Miranda-Vilela AL, Fascineli ML, Oliveira-Filho EC, Martins ES, Monnerat RG & Grisolia CK	2014b	Genotoxic evaluation in *Oreochromis niloticus* (Fish: *Characidae*) of recombinant spore–crystal complexes Cry1Ia, Cry10Aa and Cry1Ba6 from *Bacillus thuringiensis.* Ecotoxicology 23:267-272. GLP: no Published	Y	N	-	-
MMA 5.4/03	Mezzomo BP, Miranda-Vilela AL, de Souza Freire I, Barbosa LCP, Portilho FA & Grisolia CK	2013	Hematotoxicity of *Bacillus thuringiensis* as Spore-crystal Strains Cry1Aa, Cry1Ab, Cry1Ac or Cry2Aa in Swiss Albino Mice. Journal of Hematology & Thromboembolic Diseases 1(1). GLP: no Published	Y	N	-	-

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**
MMA 5.4/03	Mezzomo BP, Miranda-Vilela AL, Barbosa LCP, Albernaz VL & Grisolia CK	2015	Hematotoxicity and Genotoxicity Evaluations in Swiss Mice Intraperitoneally Exposed to *Bacillus thuringiensis* (var *kurstaki)* Spore Crystals Genetically Modified to Express Individually Cry1Aa, Cry1Ab, Cry1Ac, or Cry2Aa Environmental Toxicology 31(8):970-978. GLP: no Published	Y	N	-	-