European Commission

Draft Renewal Assessment Report prepared according to the Commission Regulation (EU) N° 1107/2009

Microbial Pest Control Agent (MPCA) Bacillus thuringiensis subsp. *kurstaki* SA-12

Volume 3 B.7 (MPCA)

Residues in or on treated products, food and feed

Rapporteur Member State: Denmark

Co- Rapporteur Member State: The Netherlands

Version history

When	What
2008	DAR
2011	Addendum to the DAR
2018	Initial RAR

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B Summary of the data and information

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

INTRODUCTION

Bacillus thuringiensis subsp. *kurstaki* SA-12 (in the following abbreviated as Btk SA-12) was one of the existing active substances covered by the Regulation (EC) No 2229/2004 on the implementation of the fourth stage of the program of work referred to in Article 8(2) of Council Directive 91/414/EEC. In Annex I to Regulation (EC) No 2229/2004 the Commission designated Denmark as rapporteur Member State to carry out the assessment of Btk SA-12 on the basis of a joint dossier submitted for the Btk strains SA-11, SA-12 and EG 2348. The notifier for Btk SA-11 and SA-12 was Mitsui AgriScience International SA/NV while EG 2348 was notified by Mitsui AgriScience International SA/NV and Intrachem Bio Italia S.p.A. (now CBC (Europe) S.r.l.). In accordance with the provisions of Article 22(1) of Regulation (EC) No 2229/2004, Denmark submitted in January and February 2008 to the EFSA the draft assessment report, including, as required, a recommendation concerning the possible inclusion of Btk SA-12 in Annex I to the Directive. The Commission examined the draft assessment report, the recommendations by the rapporteur Member State and the comments received from other Member States in consultation with experts from a certain number of Member States. The Commission referred on 12 July 2008 a draft review report to the Standing Committee on the Food Chain and Animal Health, for final examination. The draft review report was finalized in the meeting of the Standing Committee on 12 July 2008. Subsequently Regulation (EC) No 1107/2009 repealed and replaced Directive 91/414/EEC and the active substance Btk SA-12, was deemed to be approved under that Regulation and included in the Annex to Regulation (EC) No 540/2011. EFSA delivered its conclusions on *Bacillus thuringiensis* ssp. *kurstaki* (strains ABTS-351, PB-54, SA-11, SA-12, EG2348) on the 16 December 2011 (published 23 February 2012). Based on this new information available, no need to change the conditions of approval of Btk SA-12 was identified. The Commission filed on 13 December 2013 an updated review report for Btk strains SA-11, SA-12 and EG 2348 to the Standing Committee on the Food Chain and Animal Health for examination.

The approval of Btk SA-12 under the Regulation (EC) No 1107/2009 expires 30 April 2019. In accordance with the same Regulation the original notifier Mitsui AgriScience International SA/NV has filed to the Commission an application for the renewal of the approval of the active substance Btk SA-12 on 30 April 2016. In accordance with Regulation (EU) 2016/183 the notifier submitted to the designated RMS Denmark, the co-RMS The Netherlands as well as to EFSA and Commission a dossier for renewal of Btk SA-12 considering the deadline stated in SANTE-2016-10616–rev. 3.

Btk SA-12 is a wild type strain originating from infested insects. Btk acts highly specific against insect species of the order Lepidoptera and is not expected to have any harmful effects on beneficials and other non-target species of other insect orders. The insecticidal activity of Btk is mainly attributed to spore bound insecticidal pro-proteins (*Cry* toxins) which are ingested by the target pests and activated under alkaline conditions in the midgut of the larvae. The first assessment of the strain proved that it does not have any harmful effects on human or animal health or on groundwater or any unacceptable influence on the environment. The overall conclusion from EFSA (2012) confirms that no critical areas of concern are identified within the framework of the use which was supported.

As the manufacturing process of Btk SA-12 has not been changed since original approval, all data submitted for the original approval of the strain are considered fully applicable for the current evaluation.

For the renewal of the Btk strains SA-11, SA-12 and EG 2348 under Regulation (EC) 1107/2009, a separate dossier was submitted for each strain only including data, which have previously not been submitted or evaluated. Nevertheless, there is some information which is applicable to all three Btk strains, e.g. published information for Btk in general obtained during searches for peer reviewed literature according to EFSA Guidance (2011)[1] carried out for relevant sections.

In the following for ease of information, full study summaries/sections taken from the DAR (2008) or its Final Addendum (2011) are included if they are considered relevant for renewal of Btk SA-12. In order to facilitate discrimination between new data and data already evaluated during the first approval process, the headline "New Data" begins the section with data, which have previously not been submitted or evaluated. Data and their evaluations from the original DAR and addenda to the DAR are highlighted by grey background.

1 Guidance of EFSA: 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

New information 2016

A literature search covering the last 10 years was performed (Cornelese, 2016, KMA 7/01) using the keywords *Bacillus thuringiensis* subsp. *kurstaki* OR Btk AND residue AND (consumer OR food OR feed OR risk OR bacillomycin OR cereulide OR crystal protein OR cry toxin OR cytotoxin OR enterotoxin OR entomocin OR fengycin OR cytolytic protein OR iturin, OR SA12 OR SA-12 OR SA 12 OR Delfin OR Delfin Jardin OR Javelin). Both active substance and registered products were included in the search.

The relevant literature found in the search is evaluated in the chapters below.

No specific MRL was fixed for the active substance under Reg. (EC) No 396/2005, according to Art. 18(1)(b) of that Regulation, the default MRL of 0.01 mg/kg is applicable to all food products included in Annex I to that Regulation.

Cited references

Report KMA 7/01 –Cornelese A. (2016). Literature Review on *Bacillus thuringiensis* subsp. *kurstaki* SA-12 and metabolites: Residues in or on treated products, food and feed

Unpublished report,

Rep. No.: 8961516-MA-06-01

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

In this paragraph from the DAR 2008 the submitted papers concerning persistence and likelihood of multiplication are assessed.

Inactivation of *Bacillus thuringiensis* Spores by Ultraviolet and Visible light (Griego, V.M .& Spence, K.D., 1978)

As test material spores and crystal proteins of *B.thuringiensis* HD-1 (*B.thuringiensis* subsp. *kurstaki* strain HD-1) was used. Suspensions of both spores and a spore-crystal aggregate were exposed to increasing doses of light at 254 nm and to sunlight. At increasing doses of light at 254 nm the spore-crystal aggregate gave a higher survival than for the spores alone but at 1.5 x 10^5 ergs (1 joule=10^7 ergs) there were no survivors in either systems. When exposed to sunlight the half-life of both systems was about 10 minutes.

An absorbance curve for the spores also shows that absorbance is greatest about 400 nm which is in the visible spectrum.

This study supports the conclusion that both spores and crystals of *B. thuringiensis* subsp. *kurstaki* are inactivated very fast in sunlight.

The Mechanism of Sunlight Mediated Inactivation of *Bacillus thuringiensis* crystals (Pusztai. M., Fast, P., Gringoren, L., Kaplan, H., Lessard, T. & Carey, 1991)

In this study detailed photo-stability was investigated using purified δ-endotoxins crystals from *B. thuringiensis* subsp. HD-1 and HD-73. The mechanism and time course of sunlight inactivation was investigated. In this study the mechanism of inactivation is studied in details that are not relevant for the conclusions in this evaluation, and it will therefore not be summarized here. The study confirms that crystals of *B. thuringiensis* are inactivated by sunlight. In this paper other studies of the well-known sunlight mediated deactivation of *B. thuringiensis* preparations are summarized.

Plasmid-Associated Sensitivity of *Bacillus thuringiensis* to UV light (Benoit, T.G., Wilson, G.R., Bull, D.L. & Aronson, A.I., 1990)

The aim of the study was to compare sensitivity to UV light of different strains of *B. thuringiensis* with *B. cereus* as well as to find an explanation of the different sensitivity. This has no influence on the conclusions of this evaluation and will therefore not be summarized in details. The conclusion of the study was that spores and vegetative cells of *B. thuringiensis* subsp. *kurstaki* strain HD-1, HD-73 and HD-1-9 were more sensitive to UV light than spores or cells of plasmid cured *B. thuringiensis* strains or *B. cereus*.

Microbial Ecology of *B. thuringiensis*. VI. Germination of *B. thuringiensis* Spores in the Soil (Akiba, Y., 1986)

The aim was to study the possible germination of *B. thuringiensis* subsp. *thuringiensis* and subsp. *morrisoni* in three different types of soils. The soils were collected from three mulberry plantations in Japan. Spores of the *B.*

thuringiensis subspecies were inoculated into non-sterilised and sterilised soil while a mixture of vegetative cells/spores also was inoculated into non-sterilised soil. Sampling took place 0, 1, 2, 7, and 14 days after inoculation. The soil was suspended and diluted in sterile distilled water and dilutions were plated on PP and BTV medium. Total number of viable cells, number of viable vegetative cells and number of spores were counted on the two media. The soils did not contain any soil animals.

In the non-sterilised soil inoculated with spores the total count of viable cells of *B. thuringiensis* subsp. *kurstaki* gradually decreased from 10^5 to 10^4 cells/g of soil during the 14 days. No colonies of the two subspecies were formed during the period revealing that *B. thuringiensis* did not germinate in natural soil. After inoculation with spores of *B. thuringiensis* subsp. *kurstaki* in the sterilised soils the total count of viable cells (vegetative cells and spores) either increased or was unaltered. A great increase in the number of vegetative cells in all soils was observed 24 hours after inoculation and hereafter gradually decreased during the test period. When the non-sterilised soils were inoculated with the vegetative cell/spore mixture the total count of viable cells decreased from 10^6 to 3-5 x 10^4 cells/g of soil in 2 days. Thereafter, the cell number remained unaltered at about 10^4 cells/g of soil throughout the test period. At the initiation of the experiment, vegetative cells were detected at a density of 10^6 cells/g of soil; however, no viable vegetative cells existed 2 days after inoculation.

The conclusion of the study was that for both subspecies the spores were capable of germinating in sterilised soil but not in natural non-sterilised soils and that vegetative cells inoculated into natural soil disappeared in 1-2 days.

The Phylloplane as a Source of *Bacillus thuringiensis* Variants (Smith, R.A. & Gouche, G.A., 1991)

The objective of the study was to survey the phylloplanes of temperate-climate trees to determine whether *B. thuringiensis* could be recovered. In the period 1987-1989 leaves from many different kinds of trees, both conifers and deciduous trees, were sampled and analysed for the presence of *B. thuringiensis*, which was defined as any sporeforming bacillus that made a parasporal body in association with sporulation. The sampling took place in the north, west and central USA.

Three methods were used to recover *B. thurigiensis* but these methods will not be described here. Crystal proteins analysis was also performed using gel electrophoresis and enzyme immunoassay. Isolates of *B. thurigiensis* were commonly identified on the leaves. *B. thuringiensis* could be identified from up to 70 % of the trees. Some of the isolates were further characterised by analysis of crystal proteins. This analysis showed that some of the *B. thuringiensis* isolates were the subspecies *kurstaki*. Because of the common occurrence of *B. thuringiensis* the authors concluded that the isolates recovered were naturally occurring organisms rather than residues from agricultural sprays.

This study shows that *B. thuringiensis* subsp. *kurstaki* is a common organism in phylloplane of many different trees.

Environmental Persistence of *Bacillus thuringiensis* Spores Following Aerial Application (Smith, R.A. & Barry, J.W., 1998)

The objective of this study was to monitor soil and leaf populations of *B. thuringiensis* in the Mill Creek Canyon Utah, USA after aerial application with commercial products e.g. Dipel, Thuricide and Foray, containing *B. thurigiensis* subsp. *kurstaki* and to compare with two other canyons with other spray stories.

The entire canyon was sprayed three times at weekly intervals in May/June 1993. The canyon had been treated in a similar way the 4 former years, giving a total of 15 sprayings. Seven sets of soil samples were taken from 11 paired sites during the period May 1993 through June 1995. Samples were analysed for total spore count and for *B. thuringiensis* isolates with crystal proteins. Spore-forming isolates with crystal proteins were considered subspecies of *B. thuringiensis*.

Total spore counts during this period averaged 1 x 10^6 CFU/g soil, ranging from 6 x 10^5 to 20 x 10^5 CFU/g soil. *B. thuringiensis* counts for the same period averaged 1.5 x 10^5, ranging from 1 x 10^5 to 2.5 x 10^5. Isolates of *B. thuringiensis* were recovered from 64 % of the samples sites and were found in amounts of 11-27 % of the total spore-formers. The results do not indicate any proliferation of *B. thurigiensis* throughout the period but on the other side the amount of the organism was rather stable in soil even 2 years after the last spraying. In soils from Parsleys Canyon that was sprayed once 2 years before and in soils from Middle Canyon without treatment, the averaged counts for *B. thuringiensis* were 0.13 x 10^5 and 0.4 x 10^5 CFU/g soil. At these two sites *B. thuringiensis* were recovered in 25 % and 6 % of the samples, respectively.

Samples of leaves were also collected from the three canyons. The percentage of *B. thuringiensis* strains recovered from tree leaf samples was similar for three canyons, independent of history of spraying with *B. thuringiensis*. The percentage of *B. thuringiensis* strains was 69 %, 40 % and 53 % for Mill Creek, Parsleys and Middle Canyon, respectively. Genotyping of isolates of *B. thuringiensis* showed that the total number of genotypes

was respectively 9, 4 and 10 for Mill Creek, Parsleys and Middle Canyon. Of these 7, 2 and 7 strains respectively, were subsp. *kurstaki*.

This study indicates that *B. thuringiensis* subsp. *kurstaki* from commercial products does not proliferate in the soil but they did remain viable for a long period. Indigenous phylloplane *B. thuringiensis* strains did not seem to be affected in recovery frequency or in genetic diversity from the spraying with commercial products.

The field persistence of *Bacillus thuringiensis* Spores on *Cercis occidentalis* Leaves (Pinnock, D.E., Brand, R.J., Jackson, K. L. & Milstead, J.E., 1974)

The field persistence of viable spores of four *B. thuringiensis* subsp. *kurstaki* formulations (Amdal®, Biotrol® BTB 183, Thuricide® HP and Thuricide® 90TS) was measured and compared on leaves of *Cercis occidentalis* at two different sites in California, USA. For Thuricide® HP the persistence was only determined at one location. The formulation was applied by spraying. The application rate is not stated. Samples of treated leaves were collected at different times after application and the viable spore count was determined. The method is not described but there is a reference to a paper with a method description. For Thuricide HP the persistence followed a linear model and the half-life was estimated to be 1.85 days. For the other formulations the persistence followed a segmented model. The early persistence half-life 0-3 days after treatment was estimated to be 0.58-1.07 days and the later persistence half-life (after day 3) was estimated to be 1.2-2.66 days.

Occurrence and significance of *Bacillus thuringiensis* on wine grapes (Bae, S., Fleet, G.H & Heard, G.M., 2004)

Commercial preparations of *B. thuringiensis* subsp. *kurstaki* strain HD-1 Dipel®DF and Rapax®WG were sprayed four times onto grapes of different varieties during cultivation. The application rate is not stated. The grapes were cultivated at eight vineyards in New South Wales, Australia. The first treatment (100-117 days before harvest) took place just before the first sampling of grapes and the last treatment took place 5-17 days before harvest. Individual grape berries were randomly and aseptically removed from each cluster or bunch before isolation and identification. Samples were stored at 4º C and analysed within 24 hours of harvest. Samples were suspended and diluted in a sterile peptone solution. An aliquot of the dilutions was spread onto plates with count agar (PCA+cycloheximidine). The plates were incubated at 30º C for 48 h. Colonies of *B. thuringiensis* were counted and selected isolates were purified for further treatment e.g. PCR amplification.

B. thuringiensis was consistently isolated from all grape varieties throughout the cultivation period. Its population varied between 10^2-10^6 CFU/g with the higher populations in the early stage of cultivation. At the time of harvest the population varied between 10^2-10^4 CFU/g. There is not included a blank sample (a vineyard with no treatment of *B. thuringiensis*) so it is not possible to estimate the natural background population of *B. thuringiensis*. In nine different wines prepared from the treated grapes an amount of < 50 CFU/ml was found.

Persistence of *B. thuringiensis* Berliner Insecticidal Activity on Cotton Foliage, (Beegle, C.C, Dulmage, H.T., Wolfenbarger, D.A. & Martinez, E., 1981)

In this study the insecticidal activity of *B. thuringiensis* Berliner (=subsp. *kurstaki* strain HD-1) on cottons leaves was determined in 1976 and 1977. In 1976 cotton leaves were treated with different concentrations of *B. thuringiensis* subsp. *kurstaki* strain HD-1. From 8-120 hours after application both treated and untreated leaves were collected. The leaves were washed with a buffer solution. The potencies were determined for each wash by bioassay against neonatal *H.virescens* larvae. In 1977 cotton plants were treated with Dipel® (containing *B. thuringiensis* subsp. *kurstaki* strain HD-1). From 6-192 hours after application 1-day-old larvae of *T.ni* were placed on both treated and untreated leaves.

Independent of larvae and concentration the half-life was 34-47 hours. However, the authors draw the attention to the point that it can be difficult to compare half-life values of insecticidal activity with half-life values from counting of viable spores. The authors found half-life values (spore counts) between 0.2 and 22.1 days are summarized; however most lay between 0.5 days and 1.5 days.

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

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

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

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

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

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

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

Hendriksen and Hansen (2002) have investigated the same field in the period 1997-2000 for occurrence of DMU67R. The numbers have not been decreased significantly and had stabilised around 6.6 x 10^2 CFU/g. Horizontal dispersal of DMU67R was limited while some vertical dispersal took place.

Natural occurrence of Bacillus thuringiensis on Cabbage Foliage and in Insects associated with cabbage crops (Damgaard, P.H., Hansen, B.M., Pedersen, J.C & Eilenberg, J., 1997)

A study of the natural occurrence of *B. thuringiensis* on organic grown cabbage foliage was conducted. In the study leaves of cabbage (*Brassica oleracea*) and insects were sampled from an organically grown field in Denmark in two growing seasons (1992-93). There was no history of use of *B. thuringiensis* on the field. Among the colonies that correspond to *B. cereus/B. thuringiensis* morphology about 11 % was classified as *B. thuringiensis.* A total of 321 *B. thuringiensis* isolates were obtained in 1992/93. Of these isolates 150 were selected for serotyping. The typing showed that 111 of the 150 isolates could be assigned to 12 different subspecies. Not all isolates could be assigned to a subspecies. The predominant subspecies was *kurstaki* (64 %), which was found on all sampling dates. In this study the amount of CFU/g has not been determined. The authors refer to other studies where serotyping have shown that *B. thuringiensis* subsp. *kurstaki* are commonly identified in soil but never with a frequency higher than 50 %.

This study confirms what is concluded in several other studies that *B. thuringiensis* subsp. *kurstaki* is a common organism in the phyllosphere of plants.

Stability of *Bacillus thuringiensis* and *Baculovirus heliothis* on Soybean Foliage, (Ignoffo, C.M., Hostetter, D.L. & Pinnell, 1974)

A commercial preparation of *B. thuringiensis* subsp. *kurstaki* strain HD-1 was applied to soybeans at a growth stage where pods are 18.75 mm long at 1 of the 4 uppermost nodes. Rate of application was 16 x 10^9 IU/acre. One day pre-application, and at 1, 3, 7, 14, and 28 days after application (DAT), 1 leaflet of a trifoliate leaf was collected from 15 plants. Then 3 groups of 5 leaflets were washed separately with a phosphate buffered normal saline solution. Preliminary studies indicated that > 90 % of a known quantity of *B. thuringiensis* spores was washed off with this treatment. All the washings were stored at -20° C for 4 weeks and then assayed for residual spores and insecticidal activity. Previous studies indicated no loss in activity after storage under similar conditions.

Aliquots of the washings were assayed for viable spores using a standard pour-plate technique. The spore suspensions were heat-shocked at 65° C for 30 min and aliquots were plated on trypticase-soy agar medium. Counts of colonies were made both after 24 and 48 hours incubation at 30° C. The counts were average of 3-replicates/sampling.

In table B.7.1-1 the residues of *B. thuringiensis* subsp. *kurstaki* strain HD-1 per leaflet or per g/leaf are shown. As can be seen from the table the background counts of soybean foliage averaged 40-spores/g leaf or about 100

spores per leaflet. Immediately after the treatment the counts increased with a factor of 10^6. From the results given in table B.7.1-1 a half-life time of less than 24 h can be estimated. After 28 days the spore counts returned to the background level.

Table B.7.1-1. Residues of *B. thuringiensis* subsp. *kurstaki* strain HD-1 per leaflet or per g/leaf after treatment of soybean

Days after treatment	Average No. of spores x 10^3		
	Leaflet	G leaf	% of original count
Background	0.1	0.04	-
Day of treatment	118,000	47,200	100
1	10,200	4,080	8.6
3	5,000	2,000	4.2
7	18.6	7.4	<0.1
14	3.8	1.5	<0.1
21	2.2	0.0	<0.1
28	0.2	0.06	<0.1

In the same study the insecticidal activity was also determined. This loss generally followed a pattern similar to that of the spore activity. After 24 h about 65 % of the insecticidal activity was lost, and less than 1 % of the activity was present after 3 days.

Persistence of Formulations of Bacillus thuringiensis Spores and Crystals On Eastern Red Cedar Foliage in Missouri (Hostetter, D.L, Ignoffo, C.M. & Kearby, W.H, 1975)

Five trees in a natural stand of Eastern red cedar located in Missouri were treated with water (control), and the commercial formulations Thuricide and Dipel as well as Thuricide + carbon and Thuricide + carbon + molasses. The commercial formulations contain *B. thuringiensis* subsp. *kurstaki* strain HD-1. Each tree received 8.4 x 10^4 IU. Immediately after treatment, and 1, 3, 7, 14, and 28 DAT foliage was sampled from each tree. A sub-sample was washed with a phosphate-buffered normal saline solution at 300 rpm in 15 minutes. Aliquots form the resultant suspensions were bio-assayed for insecticidal activity against 24-h-old larvae of the cabbage looper. The number of viable spores/g of foliage was estimated after plating an aliquot of the wash suspensions in trypticase-soy-agar. The plates were incubated at 37° C for 18 hrs. and colony counts were made. The viable spore counts are given as an average of 3 replicates per treatment per sampling date.

Average spore counts from untreated trees were 8 x 10^2 spores/g leaf. Immediately after treatment with Thuricide and Dipel the spore counts were 4.8 x 10^6 and 8.9 x 10^6 spores/g of leaf respectively. In table B.7.1-2, the percentages of the original viable spores at the different sampling times are shown. As can be seen from the table, 24 hours after treatment less than 50 % of the applied viable spores are remaining after treatment with Thuricide and Dipel.

Table B.7.1-2. Residues of *B. thuringiensis* subsp. *kurstaki* strain HD-1 on foliage in % of original viable spore after treatment of Eastern red cedar

	% original viable spores remaining at indicated day post- treatment				
Treatment/days	1	3	7	14	28
Thuricide	48.8	37.7	4.2	2.0	0.2
Dipel	19.8	8.2	1.6	0.6	0.2

The insecticidal activity decreases in a similar pattern as the spore activity except for Dipel, that only loses 20 % of its insecticidal activity within the first 24 hours.

Persistenca de Esporas de *Bacillus thuringiensis* en Hojas de Maiz, de Frijol y en el Suelo (Sánchez-Yáñez, J.M. & Peña-Cabriales, J.J., 2000)

The paper is in Spanish, which is a language that the rapporteur does not understand. However, there is a summary in English and the figures could partly be understood.

The aim of the study was to analyse the persistence of *B. thuringiensis* spores on maize and bean leaves and in soil. An experiment was designed in which *B. thuringiensis-JM* spores isolated from an agricultural soil and

spores of *B. thuringiensis* subsp. *kurstaki* strain HD-1 (from Dipel®) were sprayed on leaves of maize and beans growing in a greenhouse. It was applied 4 times at an application rate of 5.6 x 10^5 CFU/ml. Also sterilised and non-sterilised soils were treated with the two different *B. thuringiensis* formulations. For maize, beans, and both types of soils the number of spores (CFU/g) decreased with more than 50 % within 24 hours giving a half-life time of less than 24 hours.

RMS evaluation of section from the DAR 2008	We have no remarks to the information and references referred to in the original DAR of Btk strains SA-11, SA-12 and EG2348. We find the information relevant and still valid for renewal of Btk SA-12. Generally, survival times of Bt on leaf surfaces are very short. Applied as a spray, the δ-endotoxins are rapidly degraded and endospores are rapidly inactivated when exposed to UV radiation (Griego & Spence, 1978; Pusztai et al., 1991). Minimum survival rates can be assumed on sunlight-exposed leaves, since solar radiation is the key factor in reducing persistence of populations and activity of Bt preparations, modulated by rainfall and other environmental factors on the leaf surface (Pinnock et al., 1974). The half-life of Bt spores on soybean foliage is less than 1 day, and only 8.6% of the initial population was still be viable after 1 day. Spore counts returned to background levels after 28 days on soybean (Ignoffo et al., 1974), and after 14 days on cabbage (Pedersen et al., 1995). Hostetter et al. (1975) determined the persistence of Bt spores on *Juniperus virginiana* following spraying of DiPel (containing *B. thuringiensis* ssp. *kurstaki* strain HD-1) and also found a half-life times of less than 1 day. Multiplication of Btk does not seem to play a role in natural environments (Smith et al. 1998; Akiba et al. 1986). As seen from the data on persistence presented above, no multiplication occurs on leaves due to sensitivity to solar radiation, foliage exudates and microbial competition.

New information 2016

Information on persistence in the environment, also on vegetation, is provided in Vol. 3 MA, Section B.8.

In the Scientific Opinion of the EFSA BIOHAZ panel[2] information from literature on the fate of *B. thuringiensis* on plants after application as a MPCA has been published. This document includes information from studies that were considered during the original approval of Btk SA-12 as indicated above. Furthermore, the opinion contains more recent information on residues after application. Madsen et al. (2011) were able to detect between 100 and 1000 spores/g leaf 60 days after application, on white cabbage. In fields sprayed with a product containing *B. thuringiensis* serotype *kurstaki*, 2×10^4 spores/g were found in broccoli 1 week after the spraying and 8×10^3 and 2×10^3 spores/g in celery 1 and 2 months after the spraying, respectively. In an experiment where *B. thuringiensis* spores were spread on curly kale in a field 4 times, a decrease to detection level within 30 days after the first application was found, whereas this level was not reached for the last application in 120 days (Hendriksen, 2011 taken by EFSA panel from OECD ENV/JM/MONO(2011) 42 published presentations).

The growth of endospores is initially dependent on the germination of the spore, followed by divisions of the vegetative cell. On leaves, *B. thuringiensis* occurs mainly as spores, the concentration of nutrients on the leaf surface is insufficient to mediate growth of *B. thuringiensis*. Taking into account knowledge about the fate of *B. thuringiensis* spores on leaves after spraying (i.e. the decay initially occurs rapidly and tails off thereafter), and that the decay follows the general exponential model for decay, that growth is very limited and that the spores are either directly or indirectly exposed to sunlight, it is possible to propose a model to calculate:

Population density = $I_u - k_1 \text{Exp}(I_1) - k_2 \text{Exp}(I_2) - E_r$

Where I_u is the density of spores on the plant obtained by the spraying, I_1 is the fraction of the density of spores directly exposed to sunlight on the plant and I_2 is the density of spores indirectly exposed. The ratio between I_1 and I_2 is determined by the plant species and its morphology, its age and the coverage in the field. k_1 is the decay constant for the directly exposed spores and k_2 for the indirectly exposed spores. It is not possible with today's knowledge to estimate the different parameters of this explanatory model. The model can be used for a general designation of factors of importance for fate of *B. thuringiensis* on vegetables after its use as a biopesticide.

RMS evaluation	The growth of endospores is initially dependent on the germination of the spore,

[2] EFSA SCIENTIFIC OPINION on Risks for public health related to the presence of *Bacillus cereus* and other *Bacillus* spp. including *Bacillus thuringiensis* in foodstuffs EFSA Panel on Biological Hazards (BIOHAZ), EFSA Journal 2016;14(7):4524

	followed by divisions of the vegetative cell. On leaves, *B. thuringiensis* occurs mainly as spores, the concentration of nutrients of the leaf surface is insufficient to mediate growth of *B. thuringiensis. Bacillus thuringiensis* spores may persist from days to years in soil under natural field conditions, whereas survival times of Bt on leaf surfaces are very short because they are rapidly inactivated when exposed to UV radiation. Numerous factors may have an effect on the survival of Bt in soil and on leaves: Temperature, pH, moisture, soil type, presence of micro-organisms, microbial competition and photo-degradation. Multiplication of Bt does not seem to play a role in natural environments. It is not possible with today's knowledge to estimate the different parameters of the general exponential model for decay shown above.

B.7.2 Further Information required - Exposure to consumers

B.7.2.1 Non-viable residues

Crystal proteins, the other major component in commercial Bt preparations next to spores, are not toxic to mammals. Cry and Cyt proteins are spore bound and therefore only biologically active in the presence of the microorganism. As such, the environmental risk assessment of the Cry and Cyt proteins are covered by the risk assessment of the microorganism itself. In addition, crystal proteins are very unstable when exposed to light. In soil, persistence can be influenced by biotic and abiotic factors. Overall endotoxins do not persist or accumulate in soil and are degraded rapidly. Please refer to Vol. 3 MA, section B.8, point B.8.1.1.

New information 2016

The literature search covering the last 10 years (Cornelese, 2016a) included a search for residues of known metabolites. No hits on information on metabolites were found in the search.

RMS evaluation and conclusion: Non-viable residues	Endotoxins are not toxic to mammals and does not persist or accumulate in the environment. No other non-viable residues are expected.

B.7.2.2 Viable residues

Survival of Btk is limited on plant parts exposed to the sun. Relevant information has already been provided during original approval of *Bacillus thuringiensis* subsp. *kurstaki* SA-12 and is still valid. Please refer to Vol. 3 MA, section B.8.

New information 2016

The EFSA BIOHAZ panel on Risks for public health published a Scientific Opinion related to the presence of *Bacillus cereus* and other *Bacillus* spp. including *Bacillus thuringiensis* in foodstuffs[3]. Due to lack of available data, it was not possible to conduct a quantitative evaluation of the risk to public health arising from the presence of *B. thuringiensis* in food. Therefore, a qualitative evaluation has been done, considering relevant scientific literature and information from the background documents provided by the European Commission describing the specific cases in the alleged food poisoning due to *B. thuringiensis*. An evaluation of the occurrence and levels of *B. thuringiensis* in foods has been carried out, through an extensive literature review.

The BIOHAZ panel performed an extensive literature search in order to obtain information on the presence and levels of *B. thuringiensis* in food. Information on the presence and levels of *B. thuringiensis* in food extracted from the papers included in this search is difficult to summarise because very heterogeneous types of food (raw and cooked) have been analysed and in most of the cases details on measurements are missing. Additionally, the

[3] EFSA SCIENTIFIC OPINION on Risks for public health related to the presence of *Bacillus cereus* and other *Bacillus* spp. including *Bacillus thuringiensis* in foodstuffs EFSA Panel on Biological Hazards (BIOHAZ), EFSA Journal 2016;14(7):4524

methodologies and techniques used to determine the presence and levels of *B. thuringiensis* in food samples are very diverse and in general, none of the analytical methodologies available and used in the selected research studies can be classified as 100% reliable. The levels of *B. thuringiensis* reported in food are very variable, in most cases below 10^3 CFU/g. *Bacillus thuringiensis* strains isolated from foods can in some cases be related to the use of biopesticides containing *B. thuringiensis*, but in most cases this possible relation has not been investigated.

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

In one paper (Rosenquist *et al*. (2005)), 70% (28/40) of the *B. cereus* group strains isolated from food matrices contained visible crystals and were therefore identified as *B. thuringiensis,* however it was noted that further studies are needed to clarify the genetic relationship of the isolated strains to commercial *B. thuringiensis*. The levels of *B. thuringiensis* reported in food are very variable, in most cases below 10^3 CFU/g. The majority of the food found to contain potential *B. thuringiensis* spores, were foods that would not normally be treated with *B. thuringiensis*, such as meats, milk, and pasta.

It was concluded that taking the enterotoxigenic potential into account, together with the fact that *B. thuringiensis* cannot be distinguished from *B. cereus* at the chromosomal level; the levels of *B. cereus* that can be considered as a risk for consumers are also likely to be valid for *B. thuringiensis.*

The Rosenquist paper is indicative of the ubiquitous nature and natural occurrence of *B. cereus*. No evidence was provided, nor were there any implications in this study, that any of the strains, *Bacillus thuringiensis* or *B. cereus*-like strains, were involved in any cases or outbreaks of food poisoning.

Detailed analysis of the *B. cereus*-like isolates indicated that only 10 (35.7 %) of these strains produced crystals and were positive for *cry1* genes, a characteristic that any isolate originating from a *B. thuringiensis* spray treatment would possess. Of these 10 isolates, 4 were from raw sausage, pasta, bread, and honey. These foods are not from crops that are normally treated with *B. thuringiensis* insecticides. Therefore, only 6 (15%) of the original 40 isolates selected for more detailed taxonomic analysis could have possibly had their origin from *B. thuringiensis* insecticide sprays. Importantly, these six isolates were from red pepper (2), cauliflower (1), leeks (1), salad (1), and figs (1, not usually treated), none of which are typically associated with food poisoning caused by *B. cereus* group species. It should be remembered that *B. thuringiensis* is closely related to *B. cereus* which is found ubiquitously in nature.

Hendriksen et al. (2006) investigated the appearance of residues of *Bacillus thuringiensis* subsp. *kurstaki* HD1 on retail cabbage after the use as biopesticide. Extracted kale samples were grown on petri dishes. In total, 134 *B. cereus*-like colonies were isolated. The isolates were examined by phase-contrast microscopy for their ability to produce parasporal inclusion bodies (crystals) in the sporangium. Random amplification of polymorphic DNA (RAPD) method was used, and the authors report that common RAPD pattern was found for Bt *kurstaki* HD1 and Bt *aizawai* strains HD131, HD137, HD11, HD112 and HD283, and that this pattern was not found in 22 used *B. cereus* strains. The authors reported that the maximum quantity of the Bt detected was 10^2 CFU/g cabbage. The half-life of Bt on cabbage was estimated at 16 h (Pedersen et al., 1995). Thus, even assuming that all the Bt present in the cabbage leaves originated from the biopesticide product used on the cabbage farm, the densities detected present no risk to public health.

Stephan et al. (2014) measured residues of Bt on tomato fruit after 5 times application of the commercial product XenTari. (*Bacillus thuringiensis* subspec. *aizawai*). Whole plants were treated in a greenhouse experiment with two types of tomatoes at a mean single application rate of 2.87×10^4 CFU/g fresh weight tomato (minimum 2.1×10^4 cfu/g fresh weight, maximum 4.7×10^4 cfu/g fresh weight). Fruit were sampled at different time steps just before and after the last application and after 1, 2, 3 and 7 days after the last application. For enumerating the Bt residues tomatoes were washed in sterile solution and a sample of the wash solution was plated on TSA and incubated for 20 h at 25°C. Number of CFU per gram fresh weight was calculated. From each sample location five tomatoes were analysed. The number of CFU of each tomato was enumerated in triplicate.

Results of the greenhouse experiment show a mean concentration of 63.9 CFU/g fresh weight in untreated control, with a maximal concentration of 8.7×10^2 CFU/g fresh weight, was observed throughout the entire experiment. When XenTari® was applied five-times at weekly intervals, the mean concentration of colony forming units on tomato fruits ranged between 4.9×10^4 and 8.5×10^4 CFU/g fresh weight. The concentration of Bt spores decreased during 7 days after the last application to between 46% and 77% of the initial spore concentration immediately after the last spray. Comparable results were achieved at an experiment carried out at the commercial farm. In the untreated control a maximum concentration of 1.9×10^2 CFU/g fresh weight was achieved, whereas a single application of XenTari® on the whole plant resulted in a concentration of 2.1×10^4 CFU/g fresh weight. Within one week the concentration declined to 1.3×10^4 CFU/g fresh weight.

Zhou et al. (2008) investigated treated food for the occurrence of Bt like strains and a total of 19 *Bacillus thuringiensis*-like strains were isolated. Pre-treated samples of different food items were plated and incubated. Bacterial colonies were sub-cultured and those isolates producing a parasporal body observed under light microscope were preliminary identified as *Bacillus thuringiensis* and further characterized by a serotyping test, SDS-PAGE, random amplified polymorphic DNA, and enterotoxin gene PCR analysis. Serotyping is still the most widely accepted subspecific classification method for Bt, however it is now known that strains within the same Bt serovar often do not share biochemical, genetic and toxicological attributes. SDS-PAGE analysis can provide the idea of proteins contained in the spores/crystals, but the pattern will to high extent depend on the sample preparation (for example pH so important for solubilisation of Bt crystals, and only soluble proteins are visible on SDS-PAGE gel). By no means can SDS-PAGE protein pattern serve for Bt strains identification or even grouping. As for the enterotoxin genes PCR method, it is now known that most of the *B. cereus* group strains have enterotoxin genes present on their genome and some are even expressed. In the study, a total of 19 *B. thuringiensis* strains were isolated from food and green-tea beverages. The authors reported that by using the described methods five strains isolated, two from pasteurized full fat milk and three from green-tea beverages, were indistinguishable from commercialized *B. thuringiensis* subsp. *kurstaki* isolated from biopesticides. It has to be emphasized that by using these methods most of the Bt strains are indistinguishable from commercial Bt strains, due to a high similarity among *B. cereus* group strains and even higher between different Btk strains. However, only very low levels were detected with a maximum of 3.6 CFU/mL. A summary table with 'background levels' in various commodities is presented in Vol.3 MA Section B.2, point B.2.1.2. An overview of available information on persistence of Bt strains in the environment, also on crop, is provided in Vol.3 MA Section B.8, point 8.1.

RMS evaluation and conclusion: Viable residues	The BIOHAZ panel performed an extensive literature search in order to obtain information on the presence and levels of *B. thuringiensis* in food. Information on the presence and levels of *B. thuringiensis* in food from scientific literature is difficult to summarise because very heterogeneous types of food have been analysed and in most of the cases details on measurements are missing. Additionally, the methodologies and techniques used to determine the presence and levels of *B. thuringiensis* in food samples are very diverse and in general, none of the analytical methodologies available and used in the selected research studies have a discriminatory power for identification at subspecies or strain level. The levels of *B. thuringiensis* reported in food are very variable, in most cases below 10^3 CFU/g. *Bacillus thuringiensis* strains isolated from foods can in some cases be related to the use of biopesticides containing *B. thuringiensis*, but in most cases this possible relation cannot be justified. Levels of strains introduced by applications decrease rapidly to background levels of indigenous *B. thuringiensis*.

Cited references

Report KMA 7.2.2/01 – Frederiksen, K., Rosenquist, H., Jørgensen, K., Wilcks, A., 2006. Occurrence of natural *Bacillus thuringiensis* contaminants and residues of *Bacillus thuringiensis*-based insecticides on fresh fruits and vegetables.

Published report,

Applied and Environmental Microbiology, 72(5):3435–3440

Abstract: A total of 128 *Bacillus cereus*-like strains isolated from fresh fruits and vegetables for sale in retail shops in Denmark were characterized. Of these strains, 39% (50/128) were classified as *Bacillus thuringiensis* on the basis of their content of *cry* genes determined by PCR or crystal proteins visualized by microscopy. Random amplified polymorphic DNA analysis and plasmid profiling indicated that 23 of the 50 *B. thuringiensis* strains were of the same subtype as *B. thuringiensis* strains used as commercial bioinsecticides. Fourteen isolates were indistinguishable from *B. thuringiensis* subsp. *kurstaki* HD1 present in the products Dipel, Biobit, and Foray, and nine isolates grouped with *B. thuringiensis* subsp. *aizawai* present in Turex. The commercial strains were primarily isolated from samples of tomatoes, cucumbers, and peppers. A multiplex PCR method was developed to simultaneously detect all three genes in the enterotoxin hemolysin BL (HBL) and the non-haemolytic enterotoxin (NHE), respectively. This revealed that the frequency of these enterotoxin genes was higher among the strains indistinguishable from the commercial strains than among the other *B. thuringiensis* and *B. cereus*-like strains isolated from fruits and vegetables. The same was seen for a third enterotoxin, CytK. In conclusion, the present study strongly indicates that residues of *B. thuringiensis*-based insecticides can be found on fresh fruits and vegetables and that these are potentially enterotoxigenic.

Evaluation RMS	The study is only partly acceptable. The authors concluded, the present study strongly indicates that residues of *B. thuringiensis*-based insecticides can be found on fresh fruits and vegetables and that these are potentially enterotoxigenic. However, the used typing methods were not sufficiently reliable to unequivocally identify at strain level and therefore the conclusion must be read with reservation. No matter what, it is not surprising to find low levels of the same Btk strains on fruit and vegetables, which have been treated with plant protection products containing these Btk strains. However, it should not be indicated that *B. thuringiensis*-based insecticides found on fresh fruits and vegetables are potentially enterotoxigenic and poses a risk. There are no indications that approved Bt strains used in commercial insecticides are able to produce enterotoxins at biological relevant levels.

Report KMA 7.2.2/02 – Rosenquist, H., Smidt, L., Andersen, S.R., Jensen, G.B., Wilcks, A., 2005. Occurrence and significance of *Bacillus cereus* and *Bacillus thuringiensis* in ready-to-eat food

Published report,

FEMS Microbiology Letters, 250(1), 129-136

Abstract: Among 48,901 samples of ready-to-eat food products at the Danish retail market, 0.5% had counts of Bacillus cereus-like bacteria above 10^4 cfu g^{-1}. The high counts were most frequently found in starchy, cooked products, but also in fresh cucumbers and tomatoes. Forty randomly selected strains had at least one gene or component involved in human diarrhoeal disease, while emetic toxin was related to only one *B. cereus* strain. A new observation was that 31 out of the 40 randomly selected *B. cereus*-like strains could be classified as *Bacillus thuringiensis* due to crystal production and/or content of cry genes. Thus, a large proportion of the *B. cereus*-like organisms present in food may belong to *B. thuringiensis*.

Evaluation RMS	The study was also evaluated in the DAR and found applicable and acceptable. According to the authors, a population of up to 10^3 CFU/g *B. cereus*-like cells is "satisfactory" for ready-to-eat food, and *B. thuringiensis* ssp. *kurstaki* populations would be at this level.

Report KMA 7.2.2/03 – Hendriksen, N.B., Hansen, B.M., 2006. Detection of *Bacillus thuringiensis kurstaki* HD1 on cabbage for human consumption.

Published report,

FEMS Microbiol Lett., 257(1):106–111

Abstract: The objectives of the study were to develop a specific procedure for quantification and identification of *Bacillus thuringiensis kurstaki* HD1, which is used as a biopesticide, and to quantify its presence in different kinds of cabbage for human consumption. We found that *B. thuringiensis kurstaki* HD1 can be distinguished from other *B. thuringiensis* strains by its unique random amplification of polymophic DNA-PCR pattern with the OPA9 primer and the presence of the flagellin genes, as detected by the primers FLAB1 and FLAB2. We detected from one to 100 *Bacillus cereus*-like bacteria in 10 batches of five different cabbage products for consumption. As many as 73 out of 134 isolates (53.7%) were identical with *B. thuringiensis kurstaki* HD1. The results show that *B. thuringiensis kurstaki* HD1 from biopesticides can be found in vegetables for human consumption. The authors conclude that it is unlikely that *B. thuringiensis kurstaki* HD1 occurring on cabbage products, at the densities found in their study, is of any concern in relation to public health.

Evaluation RMS	The study is applicable and acceptable. The authors concluded that it is unlikely that *B. thuringiensis kurstaki* HD1 occurring on cabbage products, at the densities found in their study, is of any concern in relation to public health.

Report KMA 7.2.2/05 – Stephan, S., Scholz-Döbelin, H., Reintges, T., Pelz, J., Jehle, J.A. Keßler, J., 2014 Investigations on residues of XenTari® (*Bacillus thuringiensis* subspec. *aizawai*) on greenhouse tomatoes.

Published report,

Journal für Kulturpflanzen 66(9): 312–318

Abstract: XenTari® (*Bacillus thuringiensis* (B.t.) subspecies *aizawai*) is an important biological plant protection agent for the control of Noctuidae larva on tomato fruits in greenhouses and belongs to the group of presumptive *Bacillus cereus* species. In general, food control agencies do not routinely differentiate between B.t. and B. cereus and a threshold of 10^5 colony forming units (cfu)/g fresh weight is applied for presumptive *B. cereus* in official food control. As no data exists on the expected residues of B.t. spores after application, residual experiments were conducted on tomatoes in greenhouses. In the greenhouse experiment, five applications of XenTari® were applied at weekly intervals. The concentration of B.t. spores on the tomato fruits ranged in all experiments between 4.9×10^4 and 8.5×10^4 cfu/g fresh weight. For single application of B.t., a maximum spore concentration of 4.7×10^4 cfu/g fresh weight was measured. None of the experiments reached the threshold for B. cereus of 1×10^5 cfu/g, although treatments were applied in a very narrow window. The findings were confirmed by additional laboratory experiments and by experiments conducted on a commercial tomato farm. To prove the degradation of B.t. spores under protected greenhouse conditions over time, a series of samples was taken after the last application over one week. Over all, the experiments demonstrated that the concentration of B.t. spores was reduced within one week to between 46% and 77% of the initial spore concentration. Therefore, in comparison to open field condition the degradation of B.t. spores under greenhouse condition was limited. When only the upper parts of the tomato plant were treated with XenTari® a distinct reduction of B.t. spores of up to 90% of B.t. spores with a concentration of 1.85 × 103 cfu/g fresh weight on the marketable tomatoes was achieved.

Evaluation RMS	The study is applicable and acceptable. The authors concluded that the experiments demonstrated that the concentration of B.t. spores was reduced within one week to between 46% and 77% of the initial spore concentration. Therefore, in comparison to open field condition the degradation of B.t. spores under greenhouse condition was limited.

Report KMA 7.2.2/06 – Zhou, G., Yan, J., Dasheng, Z., Zhou, X., Yuan, Z., 2008. The residual occurrences of *Bacillus thuringiensis* biopesticides in food and beverages.

Published report,

International Journal of Food Microbiology, 127(1–2):68–72

Abstract: In 2006, 54 pasteurized full fat milk samples, 40 ice-cream samples, and two green-tea beverage samples were analyzed and a total of 19 *Bacillus thuringiensis*-like strains were isolated, nine from seven pasteurized milks, one from an ice-cream with peach pulp and juice, and nine from two green-tea beverages. These strains were classified as *B. thuringiensis*, contained the cry1A gene and produced crystal inclusions during sporulation. All strains were characterized by a serotyping test, SDS-PAGE, random amplified polymorphic DNA, and enterotoxic gene PCR analysis. Most isolates produced bipyramidal crystals and belonged to serotypes H3a3b, H5a5b, or H7. Furthermore, two strains from pasteurized full fat milks and three strains from green-tea beverages were indistinguishable from the *B. thuringiensis* subsp. *kurstaki* strains isolated from commercial biopesticides (Kaiyan®, Qiangdi®, Lvpuan® and Sutai®), suggesting the residual occurrences of *B. thuringiensis* from biopesticides in food and beverages.

Evaluation RMS	The study is partly acceptable. The used typing methods were not sufficiently reliable to unequivocally identify at strain level and therefore the conclusion must be read with reservation. No matter what, it is not surprising to find low levels of the same Btk strains on food, which have been treated with plant protection products containing these Btk strains.

B.7.3 Summary and evaluation of residue behaviour resulting from data submitted under points 7.1 and 7.2

Considerations from the Addendum to the DAR 2011

Consideration of the need for a PHI

It is generally considered that a PHI is not required when *Bacillus thuringiensis* ssp. *kurstaki* (Btk) products are used for pest control in agriculture. The active components of commercial *B. thuringiensis* ssp. *kurstaki* preparations, spores and crystal proteins, are not toxic or pathogenic to humans, plants, and most animals except for larvae of target and on-target species belonging to the insect order *Lepidoptera*.

There were no signs of toxicity, infectivity or pathogenicity observed in rats and monkeys upon oral, inhalatory or intravenous administration of high dosages of *B. thuringiensis* ssp. *kurstaki* in acute toxicity studies. Confirming the absence of toxicity, prolonged exposure via the oral route or via inhalation did not produce any adverse effects in rats, sheep or mice. No adverse effects were observed in humans ingesting or inhaling *B. thuringiensis* ssp. *kurstaki* spores for five days, nor were adverse effects observed in humans exposed to *B. thuringiensis* ssp. *kurstaki* during/after agricultural spraying campaigns performed in Canada, New Zealand, Denmark or the USA. Adverse health effects were neither observed in workers at production sites of *B. thuringiensis* ssp. *kurstaki* products nor in greenhouse workers applying such products. Furthermore, there is no case report linking agricultural use of *B. thuringiensis* ssp. *kurstaki* with human infections although *B. thuringiensis* ssp. *kurstaki* products have been used worldwide for more than thirty years.

B. thuringiensis ssp. *kurstaki* populations occur naturally in agricultural environments without producing any adverse effects. Spores and crystal proteins are rapidly inactivated or degraded in sunlight. As *B. thuringiensis* products are applied to leaf surfaces that are exposed to the sun, as well spores as crystal proteins disappear rapidly. Levels of strains introduced by applications decrease rapidly to background levels of indigenous *B. thuringiensis*. Therefore, any residues of *B. thuringiensis* ssp. *kurstaki,* following application of Btk products used for pest control in agriculture, are not expected to be of concern or indeed relevance for human health.

Due to the absence of toxicity and the restricted persistence on above ground plant parts a PHI is not required.

Consideration of residues

Following the Opinion of the Scientific EFSA Panel on Biological Hazards on *Bacillus cereus* and other *Bacillus* spp. in food stuffs (EFSA Journal 2005) a content of 10^4 cells of *B. cereus* / g food may sometimes pose a risk for human health. This value is derived from epidemiological data and applies for enterotoxin producing strains. Based on modelling of experimental data a contamination level of 2 x 10^6 CFU/g food appears to be required to produce adverse effects in healthy humans. Also here, the given amount refers to enterotoxin producing *B. cereus* and not to bacilli in general. According to published reports foodborne poisoning caused by other *Bacillus* spp. was always linked to strong contamination of at least10^6 CFU/g but much more in most cases. A level of less than 10^6 CFU/g might be therefore considered safe for human consumption if other toxigenic bacilli than *B. cereus* are considered.

Although Btk sometimes have been found in association with foodborne illness outbreaks its actual role in the production of health symptoms has never been approved. Even if for some Btk strains toxigenic activity under *in*

vitro conditions in the laboratory has been shown, *in vivo* toxin production, has never been recorded and no adverse effects were observed in rats or rabbits administered with high dosages of toxigenic Bt strains or their crude proteins (Wilcks et al., 2006b; Itoh et al., 1991). Also in the medical literature there is no case report associating commercially used *B. thuringiensis* strains directly with food poisoning (Siegel, 2001). It appears therefore very unlikely that production of enterotoxins leading to illness in healthy humans is a general trait of Btk.

The above given minimum infective level of *Bacillus* spp. per g food refers to a total intake of at least 5 x 10^8 spores (assuming an intake of 500 g food). For *B. cereus*, the infective level (total intake per person) is considered to range from 10^5 to 10^9 CFU (EFSA Journal, 2005). This is consistently lower than the doses tested in most of the oral toxicity studies in animals submitted with the EU dossier. Due to the absence of toxicity and pathogenicity of these high dosages it appears very unlikely that the Btk strains produce enterotoxins. It has to be also noted that similar or even higher dosages of Btk have been tested in sheep, monkeys, rats and mice (10^{12} CFU/animal, Itoh et al., 1991) and that oral uptake of 10^9 CFU of Btk per day via a commercial Btk formulation did not produce adverse effects in humans (Fisher and Rosner, 1959).

Assuming worst case conditions (no degradation of Btk populations between the treatments, the complete content of Btk reaching grape plants are found on the berries) the content of viable residues upon 3 successive applications of Delfin WG at maximum application rates (1.5 kg/ha) in a vineyard was determined to be 1.68 x 10^7 CFU/g (Table IIM 6.2-1). Assuming a more realistic scenario that considers degradation of the spore populations between the spraying events, the level of viable spores after the last treatment would be 5.6 x 10^6 CFU/g. Hence, upon consumption of 500 g berries immediately after the treatment (as a worst case), this would correspond to 2.8 x 10^9 CFU, a level that is below the dosages tested in most of the acute oral toxicity studies with animals and in the range of Btk doses tested in experiments with human volunteers without causing adverse effects upon ingestion.

It can therefore be concluded that even if grapes would be consumed directly after the treatment no unacceptable risk is to be expected for human health. Hence, a PHI is not required if products containing Btk SA-11, SA-12 or EG2348 are used for pest control.

New considerations

Residual populations of *B. thuringiensis* subsp. *kurstaki* on crop may be expected after spray application. Initial decay on leaves occurs rapidly with some tailing thereafter. The growth of endospores is dependent on the germination of the spore, followed by divisions of the vegetative cell. On leaves, *B. thuringiensis* occurs mainly as spores, the concentration of nutrients of the leaf surface is insufficient to mediate growth of *B. thuringiensis*.

A number of studies monitored the occurrence of Btk on food. The cited publications report findings on fresh food of strains of Bt that are used commercially. These results have to be considered with care. In all studies the methods of identification are molecular methods that are not suitable to unequivocally distinguish closely related strains within the group of *Bacillus* spp. Moreover, in all studies, the strains from commercially known products were used as reference and therefore biased results to a large extent. The EFSA BIOHAZ panel indicates that most cases of food-borne outbreaks caused by the *B. cereus* group have been associated with concentrations above 10^5 CFU/g and that the levels of *B. cereus* that can be considered as a risk for consumers might also be valid for *B. thuringiensis*. However, this approach is not justified as pathogenic *B. cereus* strains differ significantly from commercial Bt strains in the physiological requirements, environmental behaviour and their toxigenic potential. Based on available information it can be concluded that the risk for consumers due to possible exposure of Btk SA-12 is acceptable.

RMS evaluation	The EFSA BIOHAZ panel on Risks for public health published a Scientific Opinion related to the presence of *Bacillus cereus* and other *Bacillus* spp. including *Bacillus thuringiensis* in foodstuffs[4]. Taking into account the available information, the EFSA BIOHAZ panel on Risks for public health concluded, that most cases of food-borne outbreaks caused by the *B. cereus* group have been associated with bacterial concentrations above 10^5 CFU/g food stuff. However, in some cases both emetic and diarrhoeal illness has been reported, involving concentrations between 10^3 and 10^5 CFU/g of *B. cereus*. Following these considerations, the Panel concluded that, taking the enterotoxigenic potential into account as well as that *B. thuringiensis* cannot be distinguished from *B. cereus* at the chromosomal level, the levels of *B. cereus* that can be considered as a risk for consumers are also likely to be valid for *B. thuringiensis*. There is, however, no evidence that *B. thuringiensis* has the genetic determinants for the emetic toxin cereulide. The authors of the

[4] EFSA SCIENTIFIC OPINION on Risks for public health related to the presence of *Bacillus cereus* and other *Bacillus* spp. including *Bacillus thuringiensis* in foodstuffs EFSA Panel on Biological Hazards (BIOHAZ), EFSA Journal 2016;14(7):4524

	Opinion came to the conclusion that neither the emetic toxin of *B. cereus* nor the highly cytotoxic form of CytK, namely CytK2, are produced by Bt. All other enterotoxins could potentially be produced by members of this species. Other virulence factors such as sphingomyelase or Haemolysin II have so far not been detected in Bt. It is important to notice that EFSA does not have any regulatory or enforcement powers and its role is restricted to risk assessment. It's the RMS, MSs and EU's policy-making institutions that are the risk managers, in charge of translating EFSA's scientific opinions and risk assessments into risk management measures. The opinion by EFSA (BIOHAZ) represents a thorough update of the previous EFSA opinion on *Bacillus cereus* and other *Bacillus* spp. published in 2005. In the new version specific focus has been placed on current knowledge regarding the risks and possibility to identify *Bacillus thuringiensis* in foodstuffs, an organism of which specific strains are used extensively world-wide as plant protection products. Additionally, the BIOHAZ panel was requested to specifically consider and evaluate the alleged food-borne outbreak in Germany (2012), in which a salad containing *B. thuringiensis* was suspected to be the source of the outbreak. Please refer to Vol. 3 MA, Section B.2, Point, B.2.8 and Vol. 3 MA, Section B.6, Point, B.6.1.1. The RMS finds the opinion to represent a very thorough assessment and revision of the topic with much valuable new information. We particularly find it important for scientific risk assessment to consider differences between *B. thuringiensis* at the strain level and thus to make a clear distinction between *B. thuringiensis* in general and the specific strains, approved and used as bio-pesticides. Although this point has been addressed in the new opinion (e.g. "*The levels of B. cereus group posing a health risk to consumers are highly strain-dependent due to the highly diverse pathogenic potential"*) we do not find any new information to question previous risk assessments of approved strains, which in our opinion have a longstanding proven track record for safety and should not require maximum residue levels (MRL) to be set. We do not dispute the theoretical possibility to find *B. thuringiensis* strains in nature, which are capable of causing enterotoxin production in the gut environment in humans with possible adverse effects, however we do not find any convincing evidence implicating approved biopesticidal *B. thuringiensis* strains. It is important to note that the plasmid-encoded high expression of Cry toxins in biopesticidal strains is very likely to reduce their competitive ability and infectious potential in the human gut (Raymond et al. 2017[5]). To our knowledge there is very little evidence to suggest that residues of *B. thuringiensis* as a species on foodstuff may cause illness, and even less to implicate approved biopesticide strains. This is well reflected in the EFSA opinion, which states that: "*Due to lack of available data, it is not possible to conduct a quantitative evaluation of the risk to public health arising from the presence of B. thuringiensis in food. Therefore, only a qualitative evaluation has been done…*" Therefore, the RMS based on a qualitative evaluation is questioning whether the conclusion of the Panel is justified as it mainly refers to shortcomings of clinical diagnostic methods not able to distinguish between *B. cereus* group members leading to uncertainties which role Bt actually played in the recorded outbreaks. It does not take into account that: - insecticidal Bt strains differ significantly from pathogenic *B. cereus* with regard to their physiological properties (less stress resistant spores, lower germination and growth rates, less well growing at high temperature and under microaerobic conditions), ecology and environmental behaviour (highly adapted to their insect hosts) and toxigenic properties (lower potential for surface attachment and less aggressive against human cell lines, production of lower amounts of enterotoxins in the lab) - despite a certain toxigenic potential, indicated by the presence of enterotoxin genes in their genome, there is no hint that commercial Bt strains, including strain

[5] Raymond BD, Federici B (2017). In defense of *Bacillus thuringiensis*, the safest and most successful microbial insecticide available to humanity – a response to EFSA. *FEMS Microbiology Ecology.* Volume 93, Issue 7

	SA-12, will fulfil all prerequisites required to exhibit pathogenicity in humans. This includes persistence on treated crops and harvested goods until the time of consumption, the ability to survive the gastric passage, to germinate and grow in the human intestine, to attach and invade epithelium cells and to produce the respective enterotoxins, at biological relevant levels, under these conditions. For more details, please refer to Vol. 3 MA, Section B.2, Point, B.2.8. - pathogenicity is strongly strain specific and only if there is evidence that strains are sufficiently similar with regard to properties of potential relevance for human health, a read across in the pathogenicity assessment is eventually possible (SANCO/10754/2005 rev.5, 2005). - It has to be emphasized that analytical methods used in literature references were not specific enough to distinguish strains of Bt from commercial Bt strains due to a high similarity among *B. cereus* group strains and even higher similarity between different Btk strains. From the information provided above it is clear, that a commercial Bt strain, such as Btk SA-12 can hardly be compared to a pathogenic *B. cereus* strain. Therefore, any prediction of a safety level for commercial Bt strains based on information of pathogenic *B. cereus* isolates is not reasonable. Taken together all information about Btk SA-12, the risk for consumers due to use of the strain for pest control in agricultural settings appears to be acceptable and we should not require maximum residue levels (MRL) to be set. Since the authorisation of micro-organisms is by strain level, no MRL should be set on a link to another species (*B. cereus*) and inclusion in Annex IV of Reg. (EC) No. 396/2005 is strongly supported.

B.7.4 References relied on

Several literature review reports have been provided according to the guidance of EFSA (Guidance of EFSA: 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). The aim of these reports was to provide a global overview of peer-reviewed literature concerning potential side effects of *B. thuringiensis* subsp. *kurstaki* strain SA-12.

Overview of literature reports provided according to the guidance of EFSA

Data point	Author	Year	Title	Section of RMS evaluation
KMA 2.7/12 & 3.5/06	Süß, J.	2016	Literature review on *B. thuringiensis* subsp. *kurstaki* strain SA-12: Biological properties	Vol. 3MA, B.2.10
KMA 6.1.1/07	Seehase, S.	2016	Literature review on *B. thuringiensis* subsp. *kurstaki* strain SA-12: Toxicology	Vol. 3MA, B.6.3
KMA 7.1/01	Cornelese, A.	2016a	Literature review on *B. thuringiensis* subsp. *kurstaki* strain SA-12 and metabolites: Residues in or on treated products, food and feed	Vol. 3MA, B.7.4
KMA 8.1/10	Cornelese, A.	2016b	Literature review on *B. thuringiensis* subsp. *kurstaki* strain SA-12: Fate and behaviour in the environment	Vol. 3MA, B.8.3
KMA 9/01	Schöbinger, U.	2016	Literature review on *B. thuringiensis* subsp. *kurstaki* strain SA-12: Effects on non-target organisms	Vol. 3MA, B.9.8

Literature review on *Bacillus thuringiensis* ssp. *kurstaki* SA-12, Section 1: Biological properties" (Süß, 2016)

RMS comments on the literature search: "Literature review on *B. thuringiensis* subsp. *kurstaki* strain SA-12 and metabolites: Residues in or on treated products, food and feed". (Cornelese, A., 2016; submitted in Point KMA 6.1/01)	The review was made in order to identify scientific peer-reviewed open literature on the active substance *Bacillus thuringiensis* subsp. *kurstaki* SA-12 and its metabolites which may affect the assessment on human health, animal health and/or the environment, with the special consideration of residues in or on treated products. The search strategy was based on a multi-concept approach. **Relevance criteria** • Property investigated was relevant for data requirements of Regulation (EC) 1107/2009 • Subject relevant for residues of *Bacillus thuringiensis* subsp. *kurstaki* strain SA-12 analysed on products, food and feed • Subject relevant for residues of *Bacillus thuringiensis* subsp. *kurstaki* strain SA-12 occurrence on plants • Test species/system relevant to the residues on food and feed • Application on crops and consumer risk • Relevant crop / trial location **Database searched** A search was conducted using the DIMDI database provided by the German Institute of Medical Documentation and comprised of searches in MEDLINE, BIOSIS, CAB and SCISEARCH databases **Search methods** Search strategy aimed to find all recent (from 2006 – 2016) references that are relevant for residues.

	Keywords used: The following keywords were used in the searches: Active substance *Bacillus thuringiensis* OR Btk AND *kurstaki*# AND (residue?) AND (consumer OR food OR feed OR risk OR bacillomycin OR cereulide OR crystal protein# OR cry? toxin# OR cytotoxin OR enterotoxin OR entomocin OR fengycin OR cytolytic protein# OR iturin, OR SA11 OR SA-12 OR SA 11 OR Delfin OR Delfin Jardin OR Javelin. Obtained references were first subjected to a *rapid assessment* based on title and the abstract. Summary records that appeared to be relevant passed to a second step in which a detailed assessment of full text documents was conducted. **Results of the study selection process** In total 39 references were retrieved and first subjected to a *rapid assessment* based on title and the abstract. Summary records that appeared to be relevant passed to a second step in which a detailed assessment of full text documents was conducted. In total 5 references were identified as being potentially relevant. These references were subjected to a full-text assessment in Step 2. 4 references were finally classified as relevant and supportive and are included in the dossier.
Conclusion	The literature search regarding residues of *Bacillus thuringiensis* subsp. *kurstaki* strain SA-12 was accepted as valid, both regarding inclusion of databases and use of search terms. Four references were finally classified as relevant and supportive and are included in the dossier.

Data point	Author(s)	Year	Title Owner Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KMA 7/01	Cornelese, A.	2016a	LITERATURE REVIEW ON BACILLUS THURINGIENSIS SUBSP. KURSTAKI STRAIN SA-12 AND METABOLITES: RESIDUES IN OR ON TREATED PRODUCTS, FOOD AND FEED Certis USA LLC GAB Consulting GmbH, Heidelberg, Germany Report-no.: 2281384-MA-06-01_SA-12 GLP/GEP: no Published: no	no	yes	protected	Certis USA	New data for active ingredient, not previously submitted nor evaluated
KMA 7.1/01	Griego, V.M., Spence, K.D.	1978	INACTIVATION OF *BACILLUS THURINGIENSIS* SPORES BY ULTRAVIOLET AND VISIBLE LIGHT Applied and Environmental Microbiology, 35(5): 906-910 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 7.1/02	Pusztai, M., Fast, P., Gringorten, L., Kaplan, H., Lessard, T., Carey, P.R.	1991	THE MECHANISM OF SUNLIGHT-MEDIATED INACTIVATION OF *BACILLUS THURINGIENSIS* CRYSTALS Biochem. J. 273(Pt 1)(1): 43-47 Report-no:. not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 7.1/03	Benoit, T.G., Wilson, G.R., Bull, D.L., Aronson, A.I.	1990	PLASMID-ASSOCIATED SENSITIVITY OF *BACILLUS THURINGIENSIS* TO UV LIGHT Appl. and Environ. Microbiol. 56(8): 2282-2286 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 7.1/04	Akiba, Y.	1986	MICROBIAL ECOLOGY OF *BACILLUS THURINGIENSIS* VI. GERMINATION OF *BACILLUS THURINGIENSIS* SPORES IN THE SOIL Japanese Journal of Applied Entomology and Zoology, 21(1), 76-80 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008

Data point	Author(s)	Year	Title Owner Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KMA 7.1/05	Smith, R.A., Couche, G.A.	1991	THE PHYLLOPLANE AS A SOURCE OF *BACILLUS THURINGIENSIS* VARIANTS Applied and Environmental Microbiology, 57(1): 331-315 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 7.1/06	Smith, R.A., Barry, J.W.	1998	ENVIRONMENTAL PERSISTENCE OF *BACILLUS THURINGIENSIS* SPORES FOLLOWING AERIAL APPLICATION Journal of Invertebrate Pathology 71(3): 263-267 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 7.1/07	Pinnock, D.E., Brand, R.J., Jackson, K.L., Milstead, J.E.	1974	THE FIELD PERSISTENCE OF *BACILLUS THURINGIENSIS* SPORES ON *CERCIS OCCIDENTALIS* LEAVES Journal of Invertebrate Pathology, 23(3), 341-346 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 7.1/08	Bae, S., Fleet, G.H., Heard, G.M.	2004	OCCURRENCE AND SIGNIFICANCE OF *BACILLUS THURINGIENSIS* ON WINE GRAPES International Journal of Food Microbiol. 94(3):301-312 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 7.1/09	Beegle, C.C., Dulmage, H.T., Wolfenbarger, D.A., Martinez, E.	1981	PERSISTENCE OF *BACILLUS THURINGIENSIS BERLINER* INSECTICIDAL ACTIVITY ON COTTON FOLIAGE Environ. Entomol. 10(3), 400-401 Report-no.: not applicable GLP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 7.1/10	Pedersen, J.C., Damgaard, P.H., Eilenberg, J., Hansen, B.M.	1995	DISPERSAL OF *BACILLUS THURINGIENSIS* VAR. *KURSTAKI* IN AN EXPERIMENTAL CABBAGE FIELD Canadian Journal of Microbiology, Volume 41(2): 118-125 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008

Data point	Author(s)	Year	Title Owner Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KMA 7.1/11	Hendriksen, N.B., Hansen, B.M.	2002	LONG-TERM SURVIVAL AND GERMINATION OF *BACILLUS THURINGIENSIS* VAR. *KURSTAKI* IN A FIELD TRIAL Canadian Journal of Microbiology, 48(3), 256-261 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 7.1/12	Damgaard, P.H., Hansen, B.M., Pedersen, J.C., Eilenberg, J.	1997	NATURAL OCCURRENCE OF *BACILLUS THURINGIENSIS* ON CABBAGE FOLIAGE AND IN INSECTS ASSOCIATED WITH CABBAGE CROPS Journal of Applied Microbiology, 82(2): 253-258 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 7.1/13	Ignoffo, C.M., Hostetter, D.L., Pinnell, R.E.	1974	STABILITY OF *BACILLUS THURINGIENSIS* AND *BACULOVIRUS HELIOTHIS* ON SOYBEAN FOLIAGE Environmental Entomology, 3(1), 117-119 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 7.1/14	Hostetter, D.L., Ignoffo, C.M., Kearby, W.H.	1975	PERSISTENCE OF FORMULATIONS OF *BACILLUS THURINGIENSIS* SPORES AND CRYSTALS ON EASTERN RED CEDAR FOLIAGE IN MISSOURI Journal of the Kansas Entomological Society, 48(2), 189-193 Report-no. not applicable GLP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 7.1/15	Sánchez-Yáñez, J.M., Peña-Cabriales, J.J.	2000	PERSISTANCE OF BACILLUS THURINGIENSIS SPORES ON SOIL AND MAIZE AND BEAN LEAVES (IN SPANISH WITH ENGLISH ABSTRACT) Terra, 18 (4), 325-331 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008

Data point	Author(s)	Year	Title Owner Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KMA 7.1/16	Madsen, A.M., Hansen, V.M., Meyling, N.V., Hendriksen, N.B., Winding, A., Kock, K.T., Eilenberg, J.	2011	HUMAN EKSPONERING FOR MIKROBIOLOGI-SKE BEKÆMPELSES-MIDLER, DERES NATURLIGT FOREKOMMENDE SLÆGTNINGE OG ANDRE MIKROORGANISMER Bekæmpelsesmiddelforskning fra Miljøstyrelsen, Nr. 132, 93 pp. Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated
KMA 7.2.1/01	Cornelese, A.	2016a	LITERATURE REVIEW ON BACILLUS THURINGIENSIS SUBSP. KURSTAKI STRAIN SA-12 AND METABOLITES: RESIDUES IN OR ON TREATED PRODUCTS, FOOD AND FEED Certis USA LLC GAB Consulting GmbH, Heidelberg, Germany Report-no.: 2281384-MA-06-01_SA-12 GLP/GEP: no Published: no	no	yes	protected	Certis USA	New data for active ingredient, not previously submitted nor evaluated
KMA 7.2.2/01	Frederiksen, K., Rosenquist, H., Jorgensen, K., Wilcks, A.	2006	OCCURRENCE OF NATURAL BACILLUS THURINGIENSIS CONTAMINANTS AND RESIDUES OF BACILLUS THURINGIENSIS-BASED INSECTICIDES ON FRESH FRUITS AND VEGETABLES Applied and Environmental Microbiology, 72(5): 3435-3440 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated
KMA 7.2.2/02	Rosenquist, H., Smidt, L., Andersen, S.R., Jensen, G.B., Wilcks, A.	2005	OCCURRENCE AND SIGNIFICANCE OF *BACILLUS CEREUS* AND *BACILLUS THURINGIENSIS* IN READY-TO-EAT FOOD FEMS Microbiology Letters, 250(1): 129-136 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008

Data point	Author(s)	Year	Title Owner Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KMA 7.2.2/03	Hendriksen, N.B., Munk Hansen, B.	2006	DETECTION OF BACILLUS THURINGIENSIS KURSTAKI HD1 ON CABBAGE FOR HUMAN CONSUMPTION FEMS Microbiology Letters, 257(1): 106-111 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated
KMA 7.2.2/04	Pedersen, J.C., Damgaard, P.H., Eilenberg, J., Hansen, B.M.	1995	DISPERSAL OF *BACILLUS THURINGIENSIS* VAR. *KURSTAKI* IN AN EXPERIMENTAL CABBAGE FIELD Canadian Journal of Microbiology, Volume 41(2): 118-125 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 7.2.2/05	Stephan, S., Scholz-Döbelin, H., Reintges, T., Pelz, J., Jehle, J.A. Keßler, J.	2014	INVESTIGATIONS ON RESIDUES OF XENTARI® (BACILLUS THURINGIENSIS SUBSPEC. AIZAWAI) ON GREENHOUSE TOMATOES Journal für Kulturpflanzen, 66(9) 312 - 318 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated
KMA 7.2.2/06	Zhou, G., Yan, J., Dasheng, Z., Zhou, X., Yuan, Z.	2008	THE RESIDUAL OCCURRENCES OF BACILLUS THURINGIENSIS BIOPESTICIDES IN FOOD AND BEVERAGES International Journal of Food Microbiology, 127(1-2): 68-72 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated
KMA 7.3/01	EFSA	2005	Opinion of the Scientific Panel on Biological Hazard on *Bacillus cereus* and other *Bacillus* spp in foodstuff The EFSA Journal 2005, 175, 3-45 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	Addendum to the DAR

Data point	Author(s)	Year	Title Owner Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KMA 7.3/02	Wilcks, A., Hansen, B.M., Hendriksen, N.B., Licht, T.R.	2006 b	FATE AND EFFECT OF INGESTED BACILLUS CEREUS SPORES AND VEGETATIVE CELLS IN THE INTESTINAL TRACT OF HUMAN-FLORA-ASSOCIATED RATS FEMS Immunol Med Microbiol 46:70-77 Report-no. not applicable GLP/GEP: no Published: Yes	yes	no	not protected	-	DAR 2008
KMA 7.3/03	Itoh, T., Arai, T., Hirata, I.	1991	ENTEROPATHOGENICITY OF BACILLUS THURINGIENSIS FOR HUMANS Shokubutsu Boeki 45:18-22 Report-no. not applicable GLP/GEP: no Published: yes	yes	no	not protected	-	DAR 2008
KMA 7.3/04	Siegel, J.P.	2001	THE MAMMALIAN SAFETY OF BACILLUS THURINGIENSIS-BASED INSECTICIDES Journal of Invertebrate Pathology 77, 13-21, 2001 Report-no. not applicable GLP/GEP: no Published: yes	yes	no	not protected	-	DAR 2008
KMA 7.3/05	Fisher, R., Rosner, L.	1959	INSECTICIDE SAFETY, TOXICOLOGY OF THE MICROBIAL INSECTICIDE, THUTICIDE J. Agric. Food Chem. 7(10), 686-688 Report-no. not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008