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.1 (MPCA)

Identity

Rapporteur Member State: Denmark

Co- Rapporteur Member State: The Netherlands

Version history

When	What
2008	DAR
Nov 2011	Addendum
2019	Initial RAR

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B Summary of the data and information

B.1 Identity of the micro-organism

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 Subsequently Regulation (EC) No 1107/2009 repealed and replaced Directive 91/414/EEC and the active substance Btk SA-12, was deemed to be approved under that Regulation and included in the Annex to Regulation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

B.1.1 Applicant

Applicant: Mitsui AgriScience International S.A./N.V.

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B.1.2 Producer

CONFIDENTIAL information. Please refer to Volume 4.

B.1.3 Name and species description, strain characterisation

Domain:	Bacteria
Phylum:	Firmicutes
Class:	Bacilli
Order:	Bacilliales
Family:	*Bacillaceae*
Genus:	*Bacillus*
Species:	*Bacillus thuringiensis*
Subspecies:	*kurstaki*
Strain:	SA-12

B.1.3.1 Accession number in culture collection

The strain SA-12 is deposited in the ARS Culture Collection (also known as Northern Regional Research Laboratory (NRRL), at the Microbial Properties Research Unit, National Centre for Agricultural Utilization Research, Agricultural Research Service, U.S. Department of Agriculture Peoria, Illinois 61604 USA. The Reference Number is NRRL B-30791.

B.1.3.2 Scientific name and taxonomic grouping, i.e. family, genus, species, strain, serotype, pathovar or any other denomination relevant to the micro-organism

Btk SA-12 is a wild type strain isolated from an infested insect and was not manipulated or somehow modified.

The strain SA-12 (HD-119) was originally derived from the insect *Ephestia cantella* and was deposited by H.D. Burges. *Bacillus thuringiensis* subsp. *kurstaki* is indigenous at the intended area of application.

The knowledge about phylogenetic relationships within the *B. cereus* group evolved considerably during the last 10 years. As a member of the *B. cereus*-group, Btk is closely related to *B. anthracis* and *B. cereus*. Btk strains are however phylogenetically distinguishable from *B. cereus* and *B. anthracis.* For more details please refer to Volume 3, B.2.6.

For original approval of Btk SA-12 different methods have been applied for strain identification. Please refer to point B.1.3.3 below. However, to allow an unequivocal identification of strain SA-12 an attempt was made to develop strain specific markers/primers based on the sequences of the whole genome and plasmids of strain SA-12. The developed marker has been tested for specificity with a set of *Bacillus* reference strains including Btk and potentially pathogenic *B. cereus* strains. In addition, reproducibility of the results was assessed. For Btk SA-12, two specific markers have been developed which can be used to unequivocally identify the strain. For more details, please refer to Volume 4, C.1.4.1.

Endpoint: Is the MPCA genetically modified;	Btk SA-12 is not a genetically modified strain.

B.1.3.3 Test procedures and criteria used for identification at strain level

For original approval of Btk SA-12 different methods have been applied for strain characterization and identification. Below the different methods and evaluation from the DAR 2008 and the 2011 Addendum to the DAR are presented.

The affiliation of *B. thuringiensis* subsp. *kurstaki* SA-11, SA-12 and EG-2348 to *B. cereus / B. thuringiensis* is verified according to Bergey's manual of systematic bacteriology, Vol. 2 (Sneath, 1986; publication not submitted) by standard phenotypic methods used in systematic bacteriology (Smith and Regan, 1990 a and b; Barbera, 1990).

This identification was further verified by analysis of the fatty acid composition conducted by FAME-GC using the identification software Sherlock Verison 4.5 (Strauss, 2005 a-c). The affiliation of the three strains to *B. thuringiensis* is verified by the presence of parasporal crystalline inclusion bodies and their insecticidal activity.

Flagella antigen serotyping has been used to indicate the subspecies, and it is confirmed that the serotype is 3a3b, corresponding to *B. thuringiensis* subsp. *kurstaki* for all three strains. Methods according to de Barjac (1981) and Ohba & Aizawa (1978) were used for the serotyping.

In order to verify the identity of the three strains of *B. thuringiensis* subsp. *kurstaki* the notifier has submitted data on the characterization by the four characteristics: insecticidal toxins produced, genes for insecticidal toxins, plasmid profiles and DNA fingerprint (by AFLP).

Insecticidal toxins produced

SDS-soluble proteins from purified crystals from *B. thuringiensis* subsp. *kurstaki* strains contain often two major proteins, corresponding to molecular masses of app. 130 kDa and 70 kDa respectively known as the Lepidopteran active protoxin and the mosquito and Lepidopteran active protoxin, respectively.

B. thuringiensis kurstaki SA-11 and SA-12 produce four Cry-proteins, Cry1Aa, Cry1Ab, Cry1Ac and Cry2Aa, while *B. thuringiensis kurstaki* EG2348 produces three Cry- proteins Cry1Aa, Cry1Ac and Cry2Aa. The relative amounts of the four Cry toxins can be found in Volume 4 (Yamamoto and Chen, 2006)

The three strains differ with regard to δ-endotoxin composition, notably exist differences in the relative amounts of some of the crystals. This explains why the strains SA-11, SA-12 and EG2348 and their appropriate products Delfin, CoStar and Rapax differ in their specificity and their efficacy to lepidopteran species (see B.2.1.1.2). These differences constitute the major identified differences between the strains. The RMS assess that these differences are of importance for the use of the three products, as they affect specificity and efficacy on different

target lepidoteran species, but do not influence the extrapolation between the strains in relation to risk assessment, as it is the same δ-endotoxins that are present in the three strains.

Genes for insecticidal toxins

B. thuringiensis kurstaki SA-11 and SA-12 possess the genes c*ry1Aa, cry1Ab, cry1Ac* and *cry2A*, while EG2348 possess c*ry1Aa, cry1Ac* and *cry2A*. This has been documented by PCR analysis (Yamamoto and Chen, 2006) and corresponds to the toxins present in the crystals (see above).

Plasmid profile

The analyses for the three strains were conducted in accordance with the methods of Gonzalez & Carlton (1980) and Iizuka et al. (1981) by agarose gel electrophoresis.

The analysis for *B. thuringiensis kurstaki* SA-11 revealed nine extrachromosomal elements of sizes between 4 and 60 mDa and one below 4 mDa and eight plasmids ranging from 4 to 60 mDa for SA-12 (Chen and Macuga, 1990 a and b). For B. *thuringiensis kurstaki* EG2348 ten plasmid bands are identified, eight of them between 4 and 60 mDA; the analysis reveals also that EG2348 only differs from the parental strain in the replacement of the native 69 mDA plasmid by the analogous plasmid from the donor strain (Currier et al., 1988).

RMS comment: It is not possible to verify these conclusions from the reports, due to the low quality of the photos in the figures.

DNA fingerprint

Phylogenetic relations within the *Bacillus cereus* group have been analysed by AFLP (fluorescent amplified fragment length polymorphism) (Hill et al., 2004). The AFLP technique is based on the selective PCR amplification of restriction fragments from a total digest of genomic DNA. The technique involves three steps: (i) restriction of the DNA and ligation of oligonucleotide adapters, (ii) selective amplification of sets of restriction fragments, and (iii) gel analysis of the amplified fragments. All the 24 analysed *B. thuringienisis* subsp. *kurstaki* strains analysed in the study mapped to a narrow part of branch C of Cluster 1 in the phylogenetic tree. This narrow part of Branch C of Cluster 1 consists of strains from the subspecies *B. thuringiensis* subsp. *kurstaki, entomocidus, aizawai, colmeri, tolworthi, darmstadiensis* and *alesti*. The level of discrimination within the data is at least 0.25 units in genetic distance (the genetic distance is an arbitrary unit which is specific for this dataset), as two *B. thuringiensis* subsp. *kurstaki* HD-1 strains, received independently, mapped closely with a distance about 0.25. Taking this level of discrimination into account the 24 *B. thuringiensis* subsp. *kurstaki* strains mapped into six different groups, some of the strains were identical while others were identical with strains from another subspecies.

The DNA of *Bacillus thuringiensis* subsp. *kurstaki* SA-11, SA-12 and EG2348 has also been analysed by AFLP and analysed as the data of Hill et al. (2004). As expected, this place *B. thuringiensis* subsp. *kurstaki* SA-11, SA-12 and EG2348 in Cluster 1, branch C in the dendrogram. All three mapped into the same group and were not different at the 0.25 disciminative distance level. *B. thuringiensis* subsp. *kurstaki* SA-11 could not be differentiated from *B. thuringiensis* HD263, *B. thuringiensis* HD 287 and *B. thuringiensis* HD299 by this technique (Bt HD263 and Bt HD299 are kurstaki strains while Bt HD287 is not identified at the serotype level), while SA-12 and EG2348 is identical and could not be differentiated from *B. thuringiensis aizawai* HD605. Other strains not different from these three strains at the 0.25 disciminative level are: *B. thuringiensis* subsp. *aizawai* HD860, *B. thuringiensis* subsp. *colmeri* HD847 and *B. thuringiensis aizawai* GC-91 (the active ingredient of Turex). *B. thuringiensis* subsp. *kurstaki* HD1 is not included in this group, but it is still very closely related to *B. thuringiensis* subsp. *kurstaki* SA-11, SA-12 and EG2348.

RMS evaluation	Morphological and biochemical characterization, serotyping, plasmid profiling, activity spectrum, fatty acid analysis and AFLP fingerprinting have been applied for strain characterization and identification. AFLP provides a suitable way of genetically differentiating between the strains of *Bacillus*. The strains of *B. thuringiensis* subsp. *kurstaki* SA-11, SA-12 and EG2348 can be clearly differentiated from the main group of pathogenic and toxigenic *Bacillus* strains. However, it should be noted that the AFLP bands may contain different DNA sequences even though they have the same size. The dendrograms are based on different band sizes and therefore strains identified as identical may in fact not be completely identical. However, AFLP is a typing method with a high discriminative power. The AFLP data confirm that the three strains are considered adequately similar to make extrapolations with regard to risk assessment. However, the discriminative power of AFLP typing does not allow for identification at strain

	level. It is however supposed, that it is possible to establish an identification system based on a combination of methods. Therefore, the applicant should establish and evaluate a protocol (e.g. based on a combination of methods) for unequivocal identification of *B. thuringiensis* subsp. *kurstaki* SA-11, SA-12 and EG2348 at strain level.

New Information

To allow an unequivocal identification of strain SA-12 an attempt was made to develop strain specific markers/primers based on the sequences of the whole genome and plasmids of strain SA-12. The developed marker has been tested for specificity with a set of *Bacillus* reference strains including Btk and potentially pathogenic *B. cereus* strains. In addition, reproducibility of the results was assessed by running all experiments in independent triplicates. For Btk SA-12, two specific primer pairs have been developed which can be used to unequivocally identify the strain. For more details, please refer to Volume 4, Point C.1.4.1.

RMS evaluation	For identification of Btk strain SA-12 at strain level, Single Nucleotide Polymorphism (SNP) was used to create a marker based on PCR amplification and restriction enzyme analysis. As a member of the *B. cereus*-group, Btk is closely related to *B. anthracis* and *B. cereus*. Btk strains are however phylogenetically distinguishable from *B. cereus* and *B. anthracis.*
Endpoint: Identification / detection:	Btk SA-12 are characterized by morphological and biochemical characterization, serotyping, plasmid profiling, activity spectrum, fatty acid analysis, DNA fingerprinting AFLP and cry toxin analysis. For Btk SA-12 two specific primer pairs have been developed which can be used to unequivocally identify the strain.

B.1.3.4 Common name or alternative and superseded names and code names used during the development

The various synonyms for the Btk strain SA-12 is comprised by Dively (2005) as follows:

Btk strain	**Trade name of Formulated Product**	**Code number of Technical Powder**
SA-12	CoStar WG, Deliver WG	CoStar Technical, SAN 420, ATCC – SA-12, HD-119, NRRL B-30791, Bt-19703

B.1.3.5 Relationship to known pathogens

A detailed review of papers and reviews reported in the scientific literatures, which address the relationship of the micro-organism *B. thuringiensis*, including Btk strain SA-12 to closely related species and potential for pathogenicity is provided in Volume 3MA, B.2.6.

B.1.4 Specification of the material used for manufacturing of formulated products

B.1.4.1 Content of the micro-organism

The insecticidal activity (Potency in IU / mg or SU/mg) is not correlated with the amount of CFU/g MPCA. Therefore, the active ingredient of each *Bacillus thuringiensis* subsp. *kurstaki* strain in weight is not applicable. Potency in a batch is determined by a bioassay with the target organisms *Spodoptera exigua* (SU/mg activity) and/or *Trichoplusia ni* (IU/mg activity).

Dulmage et al. (1971) developed a standardized test method to determine the potency, using *Trichoplusia ni* as test organism. At the end of the test period, activity is measured by determining the LD_{50} of the test material and comparing it with that of a standard preparation. Potency is then expressed as IU/mg.

Two samples are selected from each lot group for biotests and biochemical analyses. Potency is determined in bioassays against *Spodoptera exigua* and *Trichoplusia ni* (Chen & Hargrove, 2003).

The three *B. thuringiensis* subsp. *kurstaki* strains are produced *in vivo* by fermentation process and therefore variations in yield and bioactivity exist from batch to batch. The portion of technical material produced must be adjusted accordingly by a matrix of batches with different activity in order to obtain a uniform activity of the finished product.

The notifier submitted 5-batch analysis showing the IU/mg for five batches of SA-12 technical material (Chen, 2005). The CFU / g is evaluated by Iqbal & Chen (2005).

For further information. Please refer to Volume 4. C.1.2.1.

RMS evaluation of section from the DAR 2008 and addendum to the DAR 2011	The above information was originally evaluated in the confidential part of the DAR 2008. However, during the peer-review process it was decided that the information should be included in the addendum to the DAR. We find the information and references relevant and still valid for renewal of Btk SA-12.

New data

The production process and the quality criteria for the technical material submitted to the formulation of the end-use product have not changed since original approval (please see the certificate of the manufacturer, Chen 2016), all information submitted previously is still considered valid. However, in order to meet formal requirements, a new 5 batch analysis of the content of Btk SA-12 in technical material (International Units and Colony Forming Units) are presented in Volume 4, C.1.2.3.

RMS evaluation	To support the evaluation of the strain for renewal under Regulation (EC) 1107/2009 data on recent production batches of CoStar technical powder are provided covering determination of International Units (IU), Colony Forming Units (CFU). Minimum and maximum concentration of the MPCA from these data are given to fulfil the present data requirement. We consider CFU counts in the end-use products to be more realistic values than calculations based on data for the technical material. Based on ten batch data for CoStar WG a maximum of 5.7×10^{10} CFU/g can be established. Furthermore according to the manufacturer a minimum of 8.5×10^{9} CFU/g is required to reach the target biopotency in the end-use product.
Endpoint: Minimum and maximum concentration of the MPCA used for manufacturing of the formulated product (cfu; g/kg):	Min: 2.9×10^{10} CFU/g Max: 7.5×10^{10} CFU/g

B.1.4.2 Identity and content of impurities, additives, contaminating micro-organisms

Five batches of each strain were investigated on possible presence of bacterial pathogens as follows:

- *E. coli*	
- *Shigella, Salmonella, Staphylococcus aureus*	enteric gram+ and gram– organisms as indicators of possible fecal contamination and determination of enteric pathogens)
- *Streptococcus*	

Detection limits of microbial impurities

Test / Microbial Impurity	Result	Units
Coliform/E. coli count (petrifilm)		
Coliform (Petrifilm)	<10	CFU/g
E. coli	<10	CFU/g
Pseudomonas aeruginosa (USP)	Absent	
Salmonella (BAM) w5g	Negative	
Shigella (APHA)	Negative	
Staphylococcus aureus (USP)	Absent	
Enterococcus KF Pour Plate (APHA)	<10	CFU/g
Yeast/Mold (FDA/BAM)		
Yeast	<10	CFU/g
Mold	<10	CFU/g

There was no detection of bacterial growth after 48 hours on different selective media. No bacteria were found of critical toxicological concern (Chen 2005).

Presence of maximum level of known mammalian toxins

On all stages of fermentation starting from a slant and seed flask up to production fermenters production batches are tested for contamination and the absence of ß-exotoxin.

In the analysis of five production batches of each strain (Chen, 2005b) known mammalian toxins could not be detected. Neither the subcutaneous mouse injection nor the fly larval bioassay could show any presence of closely related pathogenic species or their toxic compounds.

The concentration control analysis of five batches of each SA-12 (Chen, 2005), confirms the constant quality of the products (see Volume 4.).

New data

Microbial contaminants

As the production process of the technical material, including fermentation of the strain, has not changed since the first evaluation (please see the certificate of the manufacturer, Chen 2016), all information submitted previously is still considered valid. However, when the strain was evaluated first, there was no specific guidance for the determination of microbial contaminants available. To meet current requirements for allowed densities of microbial contaminants in microbial plant protection products as laid down in SANCO/12116/2012-rev. 0 (September 2012), new five batch data are provided for microbial contaminants in the technical material (Rodriguez, 2016, submitted in KMA 1.4.3/01). For the complete profile of five recent production batches please refer to Volume 4, Point MA 1.2.3, Table C.1.2.3-4.

In the table below the indicator organisms which were screened for, detection limits and indication if the data are compliant with SANCO/12116/2012-rev. 0 (September 2012) are provided. Descriptions of the methods are provided in Vol. 3, Section 5 MA, Point B.5.1.7.

Table MA 1.4.2 Microbial contaminant screening

Indicator species	Method	Detection limit	SANCO/12116/2012-rev. 0 limit (compliant/not compliant)	Comment
Coliforms	BAM Chapter 4	< 10 CFU/g	< 10 CFU/g	Compliant
E. coli	USP 38 Method 62	Absence in 10 g	Absence in 1 g	Compliant
Listeria	AOAC 2004.06	Absence in 25 g	Absence in 25 g	Compliant
Salmonella	USP 38 Method 62	Absence in 10 g	Absence in 25 g	Lower amount of tested substance
Shigella	BAM Chapter 6	Absence in 25 g	Absence in 25 g	Compliant
Staphylococcus aureus	USP 38 Method 62	Absence in 10 g	Absence in 1 g	Compliant
Vibrio cholera	BAM Chapter 9	Absence in 10 g	Absence in 25 g	Lower amount of tested substance
Mold	BAM Chapter 18	Determination CFU/g	< 1000 CFU/g	Compliant
Yeast	BAM Chapter 18	Determination CFU/g	< 1000 CFU/g	Compliant
Mouse IP/SC	Internal method	10^6 CFU/mouse	-	Absence of *B. anthracis*

The tested indicator species are in compliance with those indicated in SANCO/12116/2012-rev. 0 (September 2012). Although there are some trigger values which were not fully met (*Salmonella, Vibrio*) available data are considered acceptable as they demonstrate absence of a broad range of critical contaminating microorganisms. Total aerobic and anaerobic counts have been also performed but are not presented as they would count the active ingredient Btk SA-12 also and are thus not required for spore forming bacteria.

RMS evaluation of the new data	Although there are some trigger values which were not fully met (*Salmonella, Vibrio*) available data are considered acceptable as they are in compliance with those indicated in SANCO/12116/2012-rev. 0 (September 2012). The analysis shows that contamination of the MPCA (technical grade) with human or animal pathogens was absent or below the detection limit. Metabolites of toxicological concern were also not detected.
Endpoint: Identity and content of relevant impurities, additives, contaminating organisms in the technical grade of MPCA:	No additives, no impurities expected Microbial contaminant screening: Coliforms: < 10 CFU/g *E. coli*: Absence in 10 g *Listeria*: Absence in 25 g *Salmonella*: Absence in 10 g *Shigella*: Absence in 25 g *Staphylococcus aureus*: Absence in 10 g *Vibrio cholera*: Absence in 10 g Yeast and Mold: < 1000 CFU/g

B.1.4.3 Analytical profile of batches

CONFIDENTIAL information. Please refer to Volume 4.

B.1.5 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

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 1.3.3 /01 NOT SUBMITTED	Sneath, P.H.A.	1986	ENDOSPORE-FORMING GRAM-POSITIVE RODS AND COCCI in Bergey´s Manual of Systematic Bacteriology Vol. 2. Eds. Sneath, P.H.A. Mair N.S., Sharpe, M.E: and Holt, J.G. Williams and Wilkins, pp. 1104-1207 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 1.3.3 /02	Smith, R.W., Regan, K.M.	1990a	BIOCHEMICAL AND MORPHOLOGICAL CHARACTERISTICS OF BACILLUS THURINGIENSIS SPP. KURSTAKI STRAIN SA11001C98-1-1 WITH A DISCUSSION OF STRAIN HISTORY INCLUDED [redacted] Palo Alto, CA, USA Certis USA LLC, Columbia Report-no.: 90/02/02E GLP/GEP: yes Published: no	no	no	not protected	Certis USA	DAR 2008
KMA 1.3.3 /03	Smith, R.W., Regan, K.M.	1990b	BIOCHEMICAL AND MORPHOLOGICAL CHARACTERISTICS OF BACILLUS THURINGIENSIS SPP. KURSTAKI STRAIN SA12 WITH A DISCUSSION OF STRAIN HISTORY INCLUDED [redacted] Certis USA LLC, Columbia Report-no.: 90/02/02F GLP/GEP: yes Published: no	no	no	not protected	Certis USA	DAR 2008
KMA 1.3.3 /04	Barbera, P.W.	1990	NCCLS STANDARD DISK SUSCEPTIBILITY TEST OF BACILLUS THURINGIENSIS Ecogen Incorporated, Langhorne, PA 19047 Certis USA LLC, Columbia Report-no.: EC-02 GLP/GEP: no Published: no	no	no	not protected	Certis USA	DAR 2008
KMA 1.3.3 /05	Strauss, S.	2005a	FATTY ACID COMPOSITION AND CHROMATOGRAMM OF SA11 Certis USA LLC, Columbia Report-no.: not applicable GLP/GEP: no Published: no	no	no	not protected	Certis USA	DAR 2008

KMA 1.3.3 /06	Strauss, S.	2005b	FATTY ACID COMPOSITION AND CHROMATOGRAMM OF SA12 Certis USA LLC, Columbia Report-no.: not applicable GLP/GEP: no Published: no	no	no	Not protected	Certis USA	DAR 2008
KMA 1.3.3 /07	Strauss, S.	2005c	FATTY ACID COMPOSITION AND CHROMATOGRAMM OF HD-1 Certis USA LLC, Columbia Report-no.: not applicable GLP/GEP: no Published: no	no	no	Not protected	Certis USA	DAR 2008
KMA 1.3.3 /08	de Barjac, H.	1981	IDENTIFICATION OF H-SEROTYPES OF BACILLUS THURINGIENSIS Microbial control of pest and plant diseases, Burges, H.D. ed., London, Academic press, pp. 35-43 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 1.3.3 /09	Ohba, M., Aizawa, K.	1978	SEROLOGICAL IDENTIFICATION OF BACILLUS THURINGIENSIS AND RELATED BACTERIA ISOLATED IN JAPAN Journal of Invertebrate Pathology Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 1.3.3 /10	Yamamoto T. and Chen C.-Y.	2006	DELTA-ENDOTOXIN COMPONENT ANALYSIS OF DELFIN, COSTAR AND CONDOR Certis USA LLC Report-no.: not applicable GLP/GEP: No Published: no	no	no	not protected	Certis USA	DAR 2008
KMA 1.3.3 /11	González, J.M.,Carlton, B.C.	1980	PATTERN OF PLASMID DNA IN CRYSTALLIFEROUS AND ACRYSTALLIFEROUS STRAINS OF BACILLUS THURINGIENSIS Plasmid 3, p. 92-98 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 1.3.3 /12	Iizuka, T., Faust, R.M., Travers, R.S.	1981	ISOLATION AND PARTIAL CHARACTERIZATION OF EXTRACHROMOSOMAL DNA FROM SEROTYPES OF BACILLUS THURINGIENSIS PATHOGENIC TO LEPIDOPTERAN AND DIPTERAN LARVAE BY AGAROSE GEL ELECTROPHORESIS J. Sericult. Sic. Japan, pp. 120-133 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008

KMA 1.3.3 /13	Chen, C.Y., Macuga, R.	1990a	FLAGELLA ANTIGEN SEROTYPING OF BACILLUS THURINGIENSIS SSP. KURSTAKI STRAIN SA11001C98-1-1 [redacted] Palo Alto, CA, USA Certis USA LLC, Columbia Report-no.: 90/02/12A GLP/GEP: yes Published: no	no	no	not protected	Certis USA	DAR 2008
KMA 1.3.3 /14	Chen, C.Y., Macuga, R.	1990b	FLAGELLA ANTIGEN SEROTYPING OF BACILLUS THURINGIENSIS SSP. KURSTAKI STRAIN SA12 [redacted] Palo Alto, CA, USA Certis USA LLC, Columbia Report-no.: 90/02/12B GLP/GEP: yes Published: no	no	no	not protected	Certis USA	DAR 2008
KMA 1.3.3 /15	Currier, T.C., Gawron-Burke, C. and Silver, R.S.	1988	PRODUCT CHEMISTRY AND MANUFACTURING PROCESS FOR CONDOR OF INSECTICIDE Ecogen Incorporated Ecogen Report-no.: 0022 GLP/GEP: no Published: no	no	no	not protected	Certis USA	DAR 2008
KMA 1.3.3 /16	Hill, K.K., Ticknor, L.O., Okinaka, R.T., Asay, M., Blair, H., Bliss, K.A., Laker, M., Pardington, P.E., Richardson, A.P., Tonks, M., Beecher, D.J., Kemp, J.D., Kolsto, A.-B., Wong, A.C.L., Keim, P., Jackson, P.J.	2004	FLOURESCENT AMPLIFIED FRAGMENT LENGTH POLYMORPHISM ANALYSIS OF BACILLUS ANTHRACIS, BACILLUS CEREUS AND BACILLUS THURINGIENSIS ISOLATES Applied and Environmental Microbiology, 70(2), 1068-1080. Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 1.3.4 /01	Dively, C.A.	2005	STATEMENT OF STRAIN SYNONYMS Certis USA LLC, Columbia Report-no.: not applicable GLP/GEP: no Published: no	no	no	not protected	Certis USA	DAR 2008
KMA 1.4.1 /01	Dulmage, H.T., Boening, O.P., Rehnborg, C.S., Hansen, G.D.	1971	A PROPOSED STANDARDIZED BIOASSAY FOR FORMULATIONS OF BACILLUS THURINGIENSIS BASED ON THE INTERNATIONAL UNIT Journal of Invertebrate Pathology Volume 18, pp. 240-245 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008

KMA 1.4.1 /02	Chen, C.Y., Hargrove, J.L.	2003	**Confidential information Vol. 4**	no	no	not protected	Certis USA	DAR 2008
KMA 1.4.1 /03	Chen, C.Y.	2005	**Confidential information Vol. 4**	no	no	not protected	Certis USA	DAR 2008?
KMA 1.4.1 /04	Iqbal, M., Chen, C.-Y.	2005	**Confidential information Vol. 4**	no	no	not protected	Certis USA	DAR 2008
KMA 1.4.1 /05	Chen, C.Y.	2016	**Confidential information Vol. 4**	no	yes	protected	Certis USA	New data for active ingredient, not previously submitted nor evaluated
KMA 1.4.2 /01	Chen, C.Y.	2005	**Confidential information Vol. 4**	no	yes	protected	Certis USA	In Addendum to DAR 2011