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.5 (PPP) – CoStar WG

Analytical methods

Rapporteur Member State: Denmark
Co- Rapporteur Member State: The Netherlands

Version history

When	What
2008	DAR
2011	Addendum to the DAR
2019	Initial RAR

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B Summary, evaluation and assessment of the data and information

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 11 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 11 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 Subsequently Regulation (EC) No 1107/2009 repealed and replaced Directive 91/414/EEC and the active substance Btk SA-12, was deemed to be approved under that Regulation and included in the Annex to Regulation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.

The representative formulation for renewal of the approval of Btk SA-12 under Regulation (EC) 1107/2009 is CoStar WG. CoStar WG is a WG formulation having a biopotency of 90000 IU/mg. The content of the active ingredient is 85% corresponding to a maximum of 5.7×10^{13} CFU/kg product. CoStar WG was not the representative formulation for original approval of the strain. Therefore, no data have been submitted for this formulation before. However, CoStar WG, except for the active ingredient, is identical to the representative formulation for original approval, Delfin WG, containing Btk SA-11. Also the two Btk strains are very similar with regard to their biological properties and physiological requirements. It is therefore justified to use data for Delfin WG also for the evaluation of CoStar WG. In addition, the manufacturing process of SA-12 has not been changed since original approval all data previously submitted and referring to Btk SA-12 are considered fully applicable for the current evaluation.

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 information" 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. There might be some exceptions but in this case justifications/explanations are provided.

Representative uses chosen for renewal of Btk SA-12 cover control of *Cydia pomonella* in pome fruits and *Spodoptera* spp. in ornamentals as field uses, as well as *Tuta absoluta* in tomato in the greenhouse. Both, use by professionals and non-professionals is intended. Application rates range between 1 – 2 kg with 6 subsequent applications at an interval of 7 days.

It is considered that the Critical GAP of CoStar WG chosen for the renewal of the active substance Btk SA-12 covers worst case exposure scenarios for human, non-target organisms and the environment.

Critical GAP of CoStar WG for renewal of Btk SA-12

Crop	F G or I	Pest	Application			Application rate		
			Method / Kind	Growth stage of crop	Max. number (min. interval between applications) a) per use b) per crop/ season	Kg product / ha a) max. rate per appl. b) max. total rate per crop/season	g as/ha IU/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max
Pome fruits	F	*Cydia pomonella*	Foliar spray	BBCH 67-89	a) 6 (7) b) 6 (7)	a) 1.5 b) 9.0	a) 1275 / 1.35 × 10^{11} b) 7650 / 8.1 × 10^{11}	1000-1500
Tomato	G	*Tuta absoluta*	Foliar spray	BBCH 12-89	a) 6 (7) b) 6 (7)	a) 1.0 b) 6.0	a) 850 / 9.0 × 10^{10} b) 5100 / 5.4 × 10^{11}	200-1000
Ornamentals	F	*Spodoptera* spp.	Foliar spray	BBCH 12-89	a) 6 (7) b) 6 (7)	a) 2.0 b) 12.0	a) 1700 / 1.8 × 10^{11} b) 10200 / 1.1 × 10^{12}	500-1000

Biopotency of CoStar WG: 90000 IU/mg
Max. CFU content in CoStar WG: 5.7 × 10^{13} CFU/kg

B.5 Analytical methods

B.5.1 Methods for the analysis of the preparation

B.5.1.1 Methods for the identification and the determination of the content of the micro-organism(s) in the preparation

Methods for identification of the strain are described in Vol. 3 MA, Section B.1, Point B.1.3.3 and Vol. 4, Point C.1.4.1.1. Methods for the determination of the content by use of bioassays: Vol. 4, Point C.1.2.1.

New data

An analytical method has been developed and validated for the determination of the content of active ingredient *Bacillus thuringiensis* ssp. *kurstaki* SA-12 in the formulated product CoStar WG and in aqueous dilutions obtained after suspensibility and dispersibility tests.

The following analytical method for the determination of the active substance has not previously been reviewed and is provided in support of this assessment.

Report:	KMP 5.1/01, Coranelli, S. (2011)
Title:	Analytical method VALIDATION for the determination of the active ingredient content in the formulated product CoStar and in aqueous dilutions
Document No:	Study BT120/10
Guidelines:	SANCO/3030/99, rev.4.
GLP	Yes

Material	CoStar, Batch No. 20100638, containing *Bacillus thuringiensis kurstaki* strain SA-12, content: 8.9×10^{12} CFU/kg Reference item: CoStar technical, Batch No. 2888, containing *Bacillus thuringiensis kurstaki* strain SA-12, 10.5×10^{12} spores/kg Blank Formulation: CoStar inert ingredients, Batch No. 0106-16-1 Demineralized water and Standard Water D (the same used for suspensibility and dispersibility test of the study BT 122/10 reported in MP Section 2, Point MP 2.2).
Principle of the method:	The active ingredient content was determined by colonies counting and reported as spore concentration. After a first dilution of the sample (100 times) the suspension was heat shocked for 45 min. at 65°C for killing of vegetative cells. Then sufficient dilutions were prepared to obtain 30 - 300 colonies. Namely, 0.1 mL of the already diluted dispersions were plated on nutrient agar (15 - 20 mL of agar were added). Finally, the Petri dishes were incubated at 30 ± 1°C for 24 hours and the colonies were counted. The calculation of spore/g or spore/mL was performed according to the following formula: [spore] = (dilution) x (mean number of colonies counted) / (weight or volume of sample) The method was validated with regard to linearity, precision and accuracy. For the validation of the method in aqueous dilutions (dispersions), the samples were prepared using Standard Water D.
Conclusion:	The analytical method is suitable and reliable for the determination of concentration of the number of spores of *Bacillus thuringiensis kurstaki* strain SA-12 in the product CoStar WG

and in aqueous dilutions. The method was validated by definition of the linearity, the precision and the accuracy according to the criteria set by SANCO/3030/99 rev. 4.

Validation

The following validation of the analytical method for the determination of the active substances in the product and in aqueous dilutions has not previously been reviewed and is provided in support of this assessment.

Linearity over an appropriate range:

The linearity of the response was determined by analysing five solutions of reference item (technical powder) at different concentrations in demineralized water, for the formulated product-base matrix, and in Standard Water D, for the matrix consisting of aqueous dilutions.

Results validation in the formulated product:

The results were linear within the range between 0.05 and 5 g/L. The equation of the calibration line (n = 5) was found to be $y = 4 \times 10^{10} + 2 \times 10^{9}$ (correlation coefficient 0.9982). Where x is the concentration in g/L.

Results validation in the aqueous dilution:

The results were linear within the range between 0.05 and 5 g/L. The equation of the calibration line (n = 5) was found to be $y = 3 \times 10^{10} + 3 \times 10^{9}$ (correlation coefficient 0.9977). Where x is the concentration in g/L.

The results showed that the analytical method is linear in range of interest. The correlation coefficients meet the criterion $R > 0.99$.

Representative labelled documentation

Tables containing all the data are submitted in the corresponding report.

Accuracy

The accuracy of the method was evaluated by a recovery assessment.

Results validation in the formulated product:

The mean recovery rate of samples fortified with the active ingredient (technical powder) at two concentration levels was 99.92%.

Results validation in the aqueous dilution:

The mean recovery rate of samples fortified with the active ingredient (technical powder) at two concentration levels was 101.25%.

The results showed that the analytical method is accurate according to the criteria set by SANCO/3030/99 rev. 4, for which the mean recovery accepted should be within 98.0 and 102.0% in case the nominal concentration of active ingredient is > 10%.

Repeatability (at least 5 determinations)

5 replicates independent sample determinations were prepared and measured to establish the precision of the analytical method.

Results validation of formulated product:

Mean Active ingredient content: 2.62×10^{10} spores/g ± RSD 2.34%, n = 5.

Results validation of aqueous dilution:

Mean Active ingredient content: 2.72×10^{8} spores/g ± RSD 2.54%, n = 5.

Since the concentration of the test item is 1%, the value of RSD (coefficient of variation) shall be ≤ 2.68% according to the criteria set by SANCO/3030/99 rev. 4. The method is therefore considered precise to determine the active substance both in the formulated product Agree WG and in the aqueous dilutions.

Indication as to whether outliers identified have been discarded

Outliers did not occur.

Reasons for the occurrence of outliers

Not applicable: Outliers did not occur.

Table MP 5.1-1: Summary of validation of the method for the determination of *Bacillus thuringiensis kurstaki* strain SA-12.

Reference	Linearity	Accuracy (Recoveries)	Repeatability (Precision)
KMP 5.1/01	Calculated between 0.05 and 5 g/L, where x is the concentration in g/L: In the formulated product: $y = 4 \times 10^{10} + 2 \times 10^{9}$ (correlation coefficient 0.9982) In aqueous dilutions: $y = 3 \times 10^{10} + 3 \times 10^{9}$ (correlation coefficient 0.9977)	The accuracy of the method was evaluated by a recovery assessment on two fortification levels. High recovery values were obtained: In the formulated product: 99.92% In aqueous dilutions: 101.25%	The precision was evaluated upon measuring five samples from independent weightings. A high precision, expressed as coefficient of variation was found: In the formulated product: RSD 2.34% In aqueous dilutions: 2.54%

In conclusion, the analytical method is suitable and reliable for the determination of the concentration of the numbers of spores of *Bacillus thuringiensis kurstaki* strain SA-12 in the product CoStar WG and in aqueous dilutions (dispersions). Its validation, provided in support of this application, satisfies all requirements given by SANCO/3030/99 rev.4 guideline, concerning linearity, precision and accuracy.

RMS evaluation	The validation for the analytical method for determination of the active ingredient content in the formulated product CoStar and in aqueous dilutions shows the method is suitable and reliable and satisfies all requirements given by SANCO/3030/99 rev. 4 guideline, concerning linearity, precision and accuracy

B.5.1.2 Methods to establish regular control of the preparation to show that it does not contain other organisms than the indicated ones and to establish uniform

CONFIDENTIAL information. Please refer to Vol. 4, Point C.1.4.1.3.

B.5.1.3 Methods to identify any contaminating micro-organisms of the preparation

Relevant data on microbial contaminants have been provided for the technical material. The methods are described in Vol. 3 MA, Section B.5, Point B.5.1.7.

B.5.1.4 Methods for the determination of relevant impurities or metabolites in the manufactured material, if available

Relevant data on determination of endotoxins have been provided for the technical material. The methods are described in Vol. 4 Point C.1.2.2 and C.1.4.1.4.

B.5.1.5 Methods used to determine the storage stability and shelf life of the preparation

All relevant methods used for tests of storage stability of CoStar WG are described in Vol.3 MP, Section B.2, Point B.2.7 and sub-points.

B.5.2 Methods to determine and quantify residues (viable or non-viable)

Bacillus thuringiensis subsp. *kurstaki* SA-12, such as all Btk strain currently registered at EU level was proposed for inclusion into Annex IV of Regulation (EC) 396/2005. This means that no residue definition applies to the microorganism and no MRL is set for any of the existing or intended uses. This issue, however, is still under discussion. For more information, please refer to information provided for the Btk SA-12 in Vol.3 MA in Section B.7.

B.5.3 References relied on

Please refer to point with References relied on in chapter B.5, in Volume 3 (MCPA) with regard to the evaluation of the literature search.

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
KMP 5.1/01	Coranelli, S	2011	Analytical method validation for the determination of the active ingredient content in the formulated product CoStar and in aqueous dilutions Certis USA LLC, BT120/10 Biotecnologie BT Srl, Fraz. Pantalla, Italy GLP: yes Published: no	no	Protected	New data for existing formulation, not previously submitted nor evaluated	Certis USA	Submitted for the purpose of renewal