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.8 (PPP) – CoStar WG

Fate and behaviour in the environment

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 Subsequently Regulation (EC) No 1107/2009 repealed and replaced Directive 91/414/EEC and the active substance Btk SA-12, was deemed to be approved under that Regulation and included in the Annex to Regulation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 turf 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.8 Fate and behaviour in the environment

No new information has been submitted for the fate and behaviour of CoStar WG. Previously submitted information is still valid. Furthermore, the assessment is based on the information provided for the active substance. Please see the information submitted in Vol.3 MA, Section B.8.

No risk assessment is performed for metabolites. 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. Consequently, no risk assessment is needed for metabolites.

For the purpose of a risk assessment the worst-case exposure scenario is a foliar application in pome fruits (sideward application) with up to 6 applications at a dose rate of maximum 1.5 kg product/ha (1275 g *B. thuringiensis*/ha or 8.55×10^{13} CFU/ha) in water volumes of 1000 - 1500 L/ha and a foliar application in ornamentals (downward application) with up to 6 applications at a dose rate of maximum 2.0 kg product/ha (1700 g *B. thuringiensis*/ha or 1.14×10^{14} CFU/ha) in water volumes of 500 - 1000 L/ha employed as representative uses (Critical GAP provided in introduction).

B.8.1 Persistence and multiplication

B.8.1.1 Soil

Fate and behaviour in soil

The nature of this bioinsecticide does not allow application of soil degradation studies and calculation of time weighted average concentrations, as employed for chemical substances, since 'degradation' or decline of populations of micro-organisms does not follow first order kinetics of degradation.

Based on information derived from studies and the published literature on the species *Bacillus thuringiensis* and the strain *B. thuringiensis* subsp. *kurstaki* SA-12, the environmental fate and population dynamics of this bacterium can be summarized as follows:

Bacillus thuringiensis is a ubiquitous bacterium occurring worldwide, mainly in soils as well as on insects and on plant surfaces. *B. thuringiensis* belongs to the spore forming bacteria of the family Bacilliacea. Dormant spores *of B. thuringiensis* can persist for long in the environment, but are metabolically inactive. Its application in the soil will only temporally and locally alter the natural population of the species, which will slowly return to its so called dynamic equilibrium (soil homeostasis). This is confirmed by a study by Konecka et al (2014, please refer to Vol. 3 MA, Section B.8, Point B.8.1.1. for more detail) where the number of spores in soil increased from two days to one month after application and then decreased with no spores related to the applied left after 18 months.

The persistence of Cry proteins in soil is low. Biodegradation in soil is demonstrated. DT_{50}'s of 15, 12.7 and 1.5 (24°C non-sterilised) days are derived for Cry1Ac, 9.8 days for Cr1Ab, less than 14 days for Cry1Aa and DT_{90}'s < 40 days and < 21days for Cry3Bb1.

Predicted environmental population density in soil

In order to perform a risk assessment for non-target organisms the actual population of *B. thuringiensis* subsp. *kurstaki* SA-12 spores is calculated for soil, based on the maximum accumulated application rate of 12 kg product/ha in ornamentals upon foliar application, assuming 6 treatments of 2 kg/ha and as a worst case no degradation between the multiple applications. The resultant amount of active substance will be related to the top 5 cm of soil to achieve the highest theoretical soil population. The used risk envelope covers all field uses applied for. PED values for greenhouse uses are not relevant.

For the calculation the content of 850 g a.s./kg product has been considered. In addition, the PED value is indicated in CFU/kg soil dry weight (dw), based on a maximal content of 5.7×10^{13} CFU/kg.

Assumptions:

Application rate CoStar WG:

2 kg product/ha (equivalent to 1700 g a.s./ha or 8.55×10^{13} CFU/ha)

Accumulated application rate (up to 6 treatments): 12 kg product/ha, equivalent to 10200 g a.s./ha or 6.84×10^{14} CFU/ha

incorporation into the top 5 cm layer (resulting soil volume V = 0.05 m × 10,000 m² = 500 m³)

soil density ρ of 1.5 g/cm³ (= 1.5×10^3 kg/ m³)

soil mass / ha: V × ρ = 750,000 kg soil dry weight

Plant interception is not considered in the calculation as it is generally assumed that this parameter is not applicable for microbial pest control agents and products.

The resultant actual density of viable spores of *B. thuringiensis* considering the worst-case scenario is presented in Table 8.1.1-1.

Table 8.1.1-1 Calculation of the predicted environmental density of CoStar WG and *B. thuringiensis* in soil (PED_{soil}) after 6 applications at 2 kg CoStar WG/ha in ornamentals

Accumulated application rate [kg product/ha]	**Rate [mg product/m²]**	**Soil depth [cm]**	**Bulk density [g/cm³]**	**Initial PEC related to soil depth [mg product/kg soil (dw)]**
12	1200	5.00	1.5	16.0
Accumulated application rate [kg a.s./ha]	**Rate [mg a.s./m²]**	**Soil depth [cm]**	**Bulk density [g/cm³]**	**Initial PEC related to soil depth [mg a.s./ kg soil (dw)]**
10.2	1020	5.00	1.5	13.6
Accumulated application rate [CFU/ha]	**Rate [CFU/m²]**	**Soil depth [cm]**	**Bulk density [g/cm³]**	**Initial PED related to soil depth [CFU/ kg soil (dw)]**
6.84×10^{14}	6.84×10^{10}	5.00	1.5	9.12×10^8

According to the PED_{soil} calculation the expected initial density is 16.0 mg product/kg dry soil, corresponding to 9.12×10^8 CFU/kg dry soil.

B.8.1.2 Water

Surface water

Water is not the natural habitat of *B. thuringiensis*, germination of conidia and therefore multiplication in water is not expected, since *B. thuringiensis* is no aquatic bacteria and is therefore not adapted to the conditions of the aqueous environment. Reaching aquatic environments e.g. through spray drift during application in agriculture, *B. thuringiensis* comes across unfavourable conditions (e.g. lack of nutrients, temperature) leading to a rapid decline of the population size. Thus proliferation of this bacterial species in natural water bodies is not expected to occur, and population size will decline upon hostile environmental conditions. Contamination of water with *B. thuringiensis* is a temporarily limited incidence only.

The persistence of Cry proteins in water is low, though hydrolysis seems not a major degradation route (DT_{50} 130.8 to 93.7 days for Cry1Ab protein). Biodegradation is demonstrated and microbial degradation played a key role in the dissipation of Cry1Ac toxin in water. Half-lives in the range of 10 – 15 days were derived, temperature dependent.

Predicted environmental density in natural waters

The envisaged field of use as a foliar treatment may result in contamination of adjacent surface waters by spray drift. Depending on the intended use drift values for sideward and downward application are considered. The following calculation is based on worst-case scenarios of complete accumulation of test item following 6 applications in one representative crop scenario for sideward (pome fruits) and downward (ornamentals) application, each. The used risk envelope covers all uses applied for.

Table 8.1.2-1 Calculation of the predicted environmental density of CoStar WG and *B. thuringiensis* in lentic water bodies (PED_{sw}) after 6 applications at 1 kg CoStar WG/ha in pome fruits

	Application rate[1)]	Relevant drift rate [%][2)]	Amount reaching the water	Water volume (30 cm water layer)	Initial PED_{SW}
CoStar WG	9 kg/ha	9.21	82.89 mg/m²	300 L/m²	276 µg/L
***Bacillus thuringiensis* subsp. *kurstaki* SA-12**	7.65 kg/ha	9.21	70.46 mg/m²	300 L/m²	235 µg/L
	5.13×10^{14} CFU/ha		4.72×10^{9} CFU/m²		**1.57×10^{7} CFU/L**

1) accumulated application rate, assuming no degradation between applications
2) Drift value for 6 applications in fruit crops (late)

Table 8.1.2-2 Calculation of the predicted environmental density of CoStar WG and *B. thuringiensis* in lentic water bodies (PED_{sw}) after 6 applications at 2 kg CoStar WG/ha in ornamentals (Height < 50 cm)

	Application rate[1)]	Relevant drift rate [%][2)]	Amount reaching the water	Water volume (30 cm water layer)	Initial PED_{SW}
CoStar WG	12 kg/ha	1.64	19.68 mg/m²	300 L/m²	65.6 µg /L
***Bacillus thuringiensis* subsp. *kurstaki* SA-12**	10.2 kg/ha	1.64	16.73 mg/m²	300 L/m²	55.76 µg/L
	6.84×10^{14} CFU/ha		1.12×10^{9} CFU/m²		3.74×10^{6} CFU/L

1) accumulated application rate, assuming no degradation between applications
2) Drift value for 6 applications in ornamentals (height < 50 cm)

Table 8.1.2-3 Calculation of the predicted environmental density of CoStar WG and *B. thuringiensis* in lentic water bodies (PED_{sw}) after 6 applications at 2 kg CoStar WG/ha in ornamentals (Height > 50 cm)

	Application rate[1)]	Relevant drift rate [%][2)]	Amount reaching the water	Water volume (30 cm water layer)	Initial PED_{SW}
CoStar WG	12 kg/ha	6.41	76.92 mg/m²	300 L/m²	256 µg /L
***Bacillus thuringiensis* subsp. *kurstaki* SA-12**	10.2 kg/ha	6.41	65.38 mg/m²	300 L/m²	218 µg/L
	6.84×10^{14} CFU/ha		4.38×10^{9} CFU/m²		1.46×10^{7} CFU/L

1) accumulated application rate, assuming no degradation between applications
2) Drift value for 6 applications in ornamentals (height > 50 cm)

A PED_{SW} of 3.74×10^6 CFU/L (65.6 µg product/L corresponding to 55.76 µg a.s./L) is calculated for 6 applications in ornamentals (height < 50 cm) and of 1.46×10^7 CFU/L (256 µg product/L corresponding to 218 µg a.s./L) is calculated for 6 applications in ornamentals (height > 50 cm) at 2.0 kg product/ha.

A maximum PED_{SW} of **1.57×10^7 CFU/L** (276 µg product/L corresponding to 235 µg a.s./L) is calculated for 6 applications in orchards at 1.5 kg product/ha. These values are used for the further risk assessments.

B.8.1.3 Air

From the information from the original evaluation of *B. thuringiensis* subsp. *kurstaki* SA-12 a rapid degradation of Btk in air is assumed for the following reasons: inactivation by solar radiation is a very important factor causing loss of activity and degradation of bacteria spores and δ-endotoxin crystals in the field environment. Spray drift may lead to temporal concentrations in the atmosphere before spores and crystals in finer droplets will settle out. Emanuel et al (2012; please refer to Vol. 3 MA, Section B.8, Point B.8.1.3) showed that re-aerosolisation may occur under a controlled indoor environment that simulated outdoor wind conditions. However, the fate in air for these spores will follow the same decline pattern.

B.8.2 Mobility

Ground water

From the information from the original evaluation of *B. thuringiensis* subsp. *kurstaki* SA-12 the mobility of the spores can be considered limited. Various experiments showed no movement through soil columns and no dispersion in field soils. It can thus be concluded that movement of Bt through the soil by leaching is unlikely to occur. Furthermore, as the Cry and Cyt proteins of Btk are spore bound, they are not biologically active independently of the presence of the microorganism (i.e., the risk assessment for the spores will cover any risk from the spore-bound proteins).

Only for supplemental information and not indicating that any separate assessment for the metabolites is needed the following information is included. From studies provided on the adsorption of Cry proteins to soil Kd values from 837 - 10^7 are derived indicating a strong binding to soil particles. Adsorption to soil is related to the composition of soil where a high clay content provides the highest sorption rate. Sorption of Cry toxins to soil generally follows Langmuir kinetics rather than Freundlich, though also Freundlich provided acceptable fits in one experiment ($R^2 > 0.99$). The Freundlich sorption coefficient (K_F) varied from 1.81 to 91.91 with 1/n from 0.22 to 0.62 for different (soil) minerals and temperature (please refer to Vol. 3 MA, Section B.8, Point B.8.2).

The high adsorption rates to soil together with the low persistence of Cry proteins the risk for leaching to groundwater considered to be low. Based on the relationship between sorption and degradation parameters (Boesten and van der Linden, 1991)[1] the expected leaching concentration is < 0.001 µg/L.

Drinking water

Drinking water quality is monitored by screening for microbial indicator species. Potential interference with the analytical systems for the control of the quality of drinking water according to Council Directive 98/83/EC needs to be addressed. For drinking water coliforms or *E. coli*, enterococci, and *Pseudomonas aeruginosa* are monitored.

Due to the lack of close relationship with the microorganisms listed under Directive 98/83/EC, the risk of interference is considered negligible

[1] Boesten J.J.T.I. and A.M.A. van der Linden. Modelling the influence of sorption and transformation on pesticide leaching and persistence. Journal of Environmental Quality 20(2), 1991.

B.8.3 References relied on

Please refer to point with References relied on in Vol.3 MA section B.8, point B.8.4 with regard to the evaluation of the literature search.

No new references referred to.