Renewal
Assessment Report

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

List of End-points

Rapporteur Member State: Denmark

Co- Rapporteur Member State: The Netherlands

List of end-points

Rapporteur Member State	Month and year	Microbial or Viral Agent (Name)
Denmark	**January 2019**	***Bacillus thuringiensis* subsp. *kurstaki* SA-12**

FORMAT FOR THE LISTING OF END POINTS FOR A MICROBIAL OR VIRAL PEST CONTROL AGENT (MPCA) USED IN PLANT PROTECTION

General remark:
Testing of microorganisms will often be made using specifically tailored studies. Therefore, e. g. toxicity/effects endpoints may differ from case to case. This endpoint list can therefore be seen as indicative only, to be adapted in order to fit individual cases.

Identity, Biological properties, Details of uses, Further information, and Proposed Classification and Labelling

Active microorganism:	*Bacillus thuringiensis* subsp. *kurstaki* strain SA-12
Function (*e.g.* control of fungi):	Biological insecticide
Rapporteur Member State:	Denmark
Co-rapporteur Member State:	The Netherlands
Identity of the Microbial or Viral Agent used in plant protection / Active Substance) (Regulation (EU) N° 283/2013, Annex Part B, point 1)	
Name of the organism:	*Bacillus thuringiensis* subsp. *kurstaki* SA-12
Taxonomy:	Domain: Bacteria Phylum: Firmicutes Class: Bacilli Order: Bacilliales Family: Bacillaceae Genus: *Bacillus*
Species, subspecies, strain:	Species: *Bacillus thuringiensis* Subspecies: *kurstaki* Strain: SA-12
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 unequivocal identification of strain SA-12 two specific primer pairs in combination with restriction by BveI provided a specific marker.
Culture collection:	ARS Culture Collection (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. Reference Number: NRRL B-30791.
Minimum and maximum concentration of the MPCA used	Min: 8.5×10^{12} CFU/kg MPCP (CoStar WG) Max: 5.7×10^{13} CFU/kg MPCP (CoStar WG)

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for manufacturing of the formulated product (cfu; g/kg):	
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 25 g Yeast and Mold: < 1000 CFU/g
Is the MPCA genetically modified; if so provide type of modification	Btk SA-12 is not a genetically modified strain.

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Biological properties of the microorganism (Regulation (EU) N° 283/2013, Annex Part B, point 2)

Origin and natural occurrence, Background level:	Btk as a species occurs naturally in a range of environmental compartments such as soils, plant surfaces and infected insects. Strain SA-12 was isolated from infested insects. Background populations of Btk in the environment were found in the range from 10^4 to 10^5 CFU/g in soil and 0 – 10^4 CFU/g on plants in areas not previously treated with Bt.
Target organism(s):	Lepidopteran pests (GAP: *Tuta absoluta, Cydia pomonella*, *Spodoptera littoralis*)
Mode of action:	The crystal proteins of *B. thuringiensis* must be ingested to be effective against the target insect. Upon ingestion of *B. thuringiensis* by the larvae, the crystalline inclusions dissolve in the larval midgut, releasing insecticidal crystal proteins. The activated Cry toxins interact with the midgut epithelium cells of susceptible insects. After binding to the midgut receptors, they insert into the apical membrane to create ion channels, or pores, disturbing the osmotic balance and permeability. This can result in colloid-osmotic lysis of the cells. Spore germination and proliferation of vegetative cells into the haemocoel may result in septicaemia, contributing to mortality of the insect larvae.
Host specificity:	It is generally agreed that Btk acts highly specific against members of the insect family of Lepidoptera. Some are also active against Diptera or Coleoptera. The activity spectrum of a certain strain is defined by the production of *cry* toxins. Btk SA-12 was shown to be active against lepidopteran species only.
Life cycle:	*Bacillus thuringiensis* is a ubiquitous micro-organism that colonizes a range of habitats and environments and can be found in two different stages. Under favourable conditions regarding moisture, temperature and nutrients, the basic metabolizing cell type is the vegetative cell that is actively growing and dividing. When a population of vegetative cells passes out of the exponential phase of growth, usually as a result of nutrient depletion, the differentiation of endospores begins. Endospores are formed intracellularly and are liberated after lysis of the parent cells. The transformation of dormant spores into vegetative cells can be described in three stages: (i) Activation: a reversible process that prepares the spore for germination and usually results from treatments like heating or exposure to certain chemical stimuli; (ii) Germination: the breaking of the spore stage involves the swelling, rupture of the spore coat, loss of resistance to deleterious environmental factors and increase of metabolic activity; (iii) Outgrowth: development into a vegetative cell by remerging new components from the spore coat.
Infectivity, dispersal and colonisation ability:	Spores are the form of Bt that assures survival. They can survive in soil for months and it was showed that cells and spores of Bt can also survive for 10 days in water, without altering their number. Applied as a spray on above ground leaves and fruits, endospores are rapidly inactivated and δ-endotoxins are rapidly degradable when exposed to UV-radiation. Neither cells nor spores of Bt are mobile, so their dispersal is limited. It is generally agreed that Bt is a poor competitor and does not germinate and grow extensively in the environment. Except for target insects, Btk SA-12 is not expected to colonize any non-target organism and is not infective in humans.

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Relationships to known plant, animal or human pathogens:	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.*
Genetic stability:	Culture maintenance programs ensure that only genetically unchanged and pure cultures of Btk SA-12 are used for manufacturing of the strain and the end-use product. After field or greenhouse application genetic exchange is unlikely to occur and will not lead to any adverse effects. From the literature search for Btk SA-12 it can be concluded, that transfer of genetic material cannot be completely ruled out upon use of the strain as pest control agent in agricultural settings but the likelihood is rather low because the event requires germination and growth of the applied SA-12 spores at a high level and the presence of competent recipient vegetative cells at a high level. Even under these conditions, rates of genetic exchange were shown to be extremely low. In addition, Btk SA-12 is a wild type strain and does not have the capacity to produce any other compounds than indigenous Btks already present in the environment and it is not multi-resistant. Hence, in the unlikely case that genetic material would be transferred from SA-12 to indigenous bacteria, there is no risk that any unwanted properties are spread in the environment.
Information on the production of relevant metabolites (especially toxins):	Btk SA-12 produces Cry1A and Cry2A insecticidal proteins. Apart from the Cry proteins several other insecticidal proteins are produced by Bt (vegetative insecticidal proteins VIP, cytolytic proteins Cyt etc.). Absence of toxicity to humans and mammals from all metabolites involved in the mode of action was confirmed by a literature search. Beta-exotoxins, are considered to have toxic properties but were shown not to be produced by commercial Btk strains. The ability to produce *B. cereus*-enterotoxins and possible consequences for consumers is discussed since first evaluation of the strain. However, based on available knowledge on Btk including Btk SA-12, there is no hint that the strain has the ability to cause foodborne disease as it will not fulfil all prerequisites required for pathogenic action in humans.
Resistance/ sensitivity to antibiotics / anti-microbial agents used in human or veterinary medicine:	Btk SA-12 has been shown to be sensitive to a broad range of antibiotics commonly used in human and veterinary medicine. The strain is not multi-resistant.

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Summary of uses supported by available data (Regulation (EU) N° 283/2013, Annex Part B, point 3)

PPP (product name/code): CoStar WG

Formulation type: WG

Active Substance: *Bacillus thuringiensis* subsp. *kurstaki* SA-12

Conc. of a.s.: 850 g/kg or 90,000 IU/mg, min. 8.5×10^{12} CFU/kg (nom/max. 5.7×10^{13} CFU/kg)

Safener: -

Conc. of safener: -

Synergist: -

Conc. of synergist: -

Applicant: Mitsui AgriScience International SA/NV

professional use ☒

Zone(s): EU

non-professional use ☒

Verified by RMS: n

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1	2	3	4	5	6	7	8	9	10	11	12	13
					Application			Application rate				
Use-No.	Member state(s)	Crop and/ or situation (crop destination / purpose of crop)	F G or I	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Method / Kind	Timing / Growth stage of crop & season	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 a.s./ha IU/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max	PHI (days)	Remarks
1	EU	Pome fruits (apple, pear)	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	-	-
2	EU	Solanaceous fruits (tomato, aubergine, sweet pepper)	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	-	-
3	EU	Ornamentals	F	*Spodoptera littoralis*	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	-	-

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Further information, Efficacy

Effectiveness (Regulation (EU) N° 284/2013, Annex Part A, point 6.2)

	According to the latest guidance on the preparation of dossiers for the renewal of active substances, information on efficacy is not required (SANCO/10181/2013 – rev. 2.1, 13 May 2013). The representative products have all been authorised at Member State level for > 10 years and have therefore been assessed in line with Uniform Principles. The GAP for the representative uses is realistic.

Adverse effects on field crops (Regulation (EU) N° 284/2013, Annex Part A, point 6.4)

	The representative products have all been authorised at Member State level for > 10 years and have therefore been assessed in line with Uniform Principles. No unacceptable adverse effects are known.

Observations on other undesirable or unintended side-effects (Regulation (EU) N° 284/2013, Annex Part A, point 6.5)

	The representative products have all been authorised at Member State level for > 10 years and have therefore been assessed in line with Uniform Principles. No unacceptable side effects are known.

Classification and proposed labelling (Symbol, Indication of danger, Risk phrases, Safety phrases)

with regard to physical/chemical data:	Not required
with regard to toxicological data:	P102: Keep out of reach of children P501: Dispose of the container/contents in accordance with municipal rules for disposal of waste <u>Safety precaution phrases:</u> Contains *Bacillus thuringiensis* subsp. *kurstaki* SA-12; may have the potential to provoke sensitising reactions. Keep away from food, drink and animal feeding stuffs.
with regard to fate and behaviour:	Not required
with regard to ecotoxicological data:	Not required

Methods of analysis (Regulation (EU) N° 283/2013, Annex Part B, point 4 and Regulation (EU) N° 284/2013, Annex Part B, point 5)

Analytical methods for the microorganism (MA 4.1 & MP 5.1)

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Manufactured microorganism (principle of method):	Biopotency (bioassay with *T. ni*), CFU (validated method)
Impurities and contaminating microorganisms in manufactured material (principle of method):	Standard microbiological methods for detection of microbial contaminants
Microbial Pest Control Product (principle of method):	See above

Analytical methods for residues (viable and non-viable) in exposed compartments and organisms
(MA 4.2 & MP 5.2)

of the active microorganism (principle of method):	Not required as Btk SA-12 is proposed for inclusion into Annex IV of Regulation (EC) No 396/2005. However, specific markers are available to monitor the strain in agricultural fields.
of relevant metabolites (principle of method):	Cry1Ab Soil: extraction with phosphate buffered saline Tween, quantification with commercial ELISA kit. LOQ 0.25 ng/mL. Water: processing via lyophilization and filter centrifugation, quantification with ELISA. Method detection limit 2.1 ng/L.

Impact on Human and Animal Health (Regulation (EU) N° 283/2013, Annex Part B, point 5 and Regulation (EU) N° 284/2013, Annex Part B, point 7)

Medical data: (including medical surveillance on manufacturing plant personnel) (MA 5.1.1))	There are no confirmed case reports linking agricultural use of plant protection products based on Btk strains with human disease although Btk products have been used worldwide for more than sixty years. No incidents related to adverse health effects such as toxicological effects, allergic response, or irritation, to employees, resulting from exposure to *B. thuringiensis* subsp. *kurstaki* SA-12 during production and packaging of the product have been reported.
Sensitisation: (MA 5.2.1 & MP 7.2.3)	Not sensitizing
Acute oral infectivity, toxicity and pathogenicity: (MA 5.2.2.1 & MP 7.1.1)	No signs of toxicity, pathogenicity or infectivity have been detected upon single oral exposure to Btk SA-12 or a liquid formulation of Btk SA-12. LD_{50} rat > 5.4×10^8 CFU/animalcorresponding to 2.7×10^9 CFU/kg bw
Acute intratracheal/inhalation infectivity, toxicity and pathogenicity: (MA 5.2.2.2 & MP 7.1.2)	No toxicity, infectivity and pathogenicity upon pulmonary exposure observed. LC_{50} > 1.35×10^8 CFU/animal
Acute intravenous/intraperitonial infectivity: (MA 5.2.2.3)	There is no evidence that Btk SA-12 acts toxic or pathogenic following intravenous administration. LD_{50} rat > 9×10^7 CFU/animal
Genotoxicity:	No validated methods available for microorganisms.

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(MA 5.2.3)	
Cell culture study: (MA 5.2.4)	Btk is not an intracellular replicating micro-organisms, cell culture studies are not required.
Information on short-term toxicity and pathogenicity: (MA 5.2.5)	Acute toxicity studies did not reveal any signs of toxicity or pathogenicity, thus, there is no evidence that Btk SA-12 acts toxic or pathogenic following short-term exposure.
Dermal toxicity: (MP 7.1.3)	No adverse effects observed.
Specific toxicity, pathogenicity and infectivity: (MA 5.3)	CoStar WG does not require classification with regard to skin or eye irritation.
Genotoxicity – *in vivo* studies in germ cells: (MA 5.5)	No indications of genotoxicity are known for Btk.

Reference values

AOEL:	Not applicable
ADI:	Not applicable
ARfD:	Not applicable

Exposure (operator, workers, bystander, consumer): (MA 6.1 & MP 7.3, 8.0)	A qualitative risk assessment for operators, workers and residents was performed. Comparison to no-effect levels demonstrates sufficient margins of exposure. No risk is anticipated.

Residues (Regulation (EU) N° 283/2013, Annex Part B, point 6 and Regulation (EU) N° 284/2013, Annex Part B, point 8)

Viable residues:	*B. thuringiensis ssp. kurstaki* spores and crystal proteins, are not toxic or pathogenic to humans, plants, and most animals. Spores are not persistent on crop, half-life less than 1 day.
Non-viable residues:	Crystal proteins are unstable when exposed to light. half-life for insecticidal activity on leaves was 34 to 47 hours following application DT_{50} of crystal protein 24 hours after exposure to sunlight

Fate and Behaviour in the Environment (Regulation (EU) N° 283/2013, Annex Part B, point 7 and Regulation (EU) N° 284/2013, Annex Part B, point 9)

Persistence and multiplication (competitiveness) in soil, water and air:	<u>Btk SA-12</u> **Soil**: *Bacillus thuringiensis* occurs naturally and ubiquitously in the environment. It is a common component of the soil micro-biota and has been isolated from most terrestrial habitat. Available information indicates that *Bacillus thuringiensis* spores may persist from days to years in soil

	under natural field conditions. The low potential for spore germination, growth and re-sporulation in soils minimises multiplication. **Water**: *B. thuringiensis* is not regarded as an autochthonous inhabitant of aquatic environments and does not find optimal conditions for growth. Therefore, proliferation is not likely to occur. **Air**: re-aerolisation of applied spores is possible but spores rapidly drop in viability following release to air. Fate and transport via air after application is unlikely to play a role in environmental exposure to *B. thuringiensis* subsp. *kurstaki* spores and endotoxins. Endotoxins **Soil**: Persistence can be influenced by biotic and abiotic factors. Overall the results indicate that the endotoxins does not persist or accumulate in soil and is degraded rapidly (DT_{50} < 2 weeks). **Water**: Persistence can be influenced by biotic and abiotic factors. Overall the results indicate that the endotoxins does not persist or accumulate in water.
Mobility:	Btk SA-12 Mobility of spores of *B. thuringiensis* subsp. *kurstaki* SA-12 can be considered limited. Endotoxins Cry proteins are strongly adsorbed by soil and will be effectively immobilized after their release into soil.

Effects on non-target organisms (Regulation (EU) N° 283/2013, Annex Part B, point 8 and Regulation (EU) N° 284/2013, Annex Part B, point 10)

Effects on birds and mammals

Application rate (g a.s./ha)	Test substance	Crop	Category (*e.g.* insectivorous bird) and species	Time-scale	Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects)
850 - 1700	Thuricide SC (Btk SA-12)	Pome fruits, solanaceous fruits, ornamentals	Acute oral, Japanese quail	30-d observation	$LD_{50} > 5.0 \times 10^9$ CFU/kg bw/day
	Thuricide SC (Btk SA-12)		Acute oral, Rat	21-d observation	$LD_{50} > 5.4 \times 10^8$ CFU/animal corresponding to 2.7×10^9 CFU/kg bw
	CoStar Technical Concentrate (Btk SA-12)		Acute oral, Rat	14-d observation	LD_{50} > 5050 MPCP mg/kg bw ($LD_{50} > 2 \times 10^{11}$ CFU/kg bw)

Effects on aquatic organisms

Group	Test substance	Time-scale	Toxicity, infectivity and pathogenicity (endpoint, value or other description

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			of effects)
Laboratory tests			
Fish species (specify): *Danio rerio*	Thuricide SC (Btk SA-12)	30-d (semi-static)	$LC_{50} > 5.0 \times 10^9$ CFU/L
Fish species (specify): *Oncorhynchus mykiss*	CoStar WG (Btk SA-12)	96-hour (static limit)	LC_{50} >51 mg /L corresponding to 4.7×10^8 CFU/L
Invertebrate species			
Invertebrate species: (specify) *Daphnia magna*	Thuricide SC (Btk SA-12)	21-d (semi-static)	$EC_{50} > 1.0 \times 10^9$ CFU/L
Invertebrate species: (specify) *Daphnia magna*	CoStar WG[a)]	48-h (static)	EC_{50} > 141 mg/L corresponding to 1.3×10^9 CFU/L

Effects on algae: (species, growth, growth rate, capacity to recover)	*Desmodesmus subspicatus* CoStar WG[a)] 72-h (static) $EC_{50} > 696$ mg/L corresponding to 6.5×10^9 CFU/L
Effects on aquatic plants (species, growth, growth rate, capacity to recover)	Not toxic, pathogenic or infective to aquatic plants based on available experience with Btk SA-12 products and efficacy testing.

Effects on bees

Species	Crop	Test Substance	Route/time-scale	Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects)
Laboratory Test				
Apis mellifera	Pome fruits, tomatoes, ornamentals	Delfin WG (Btk SA-11)	Oral/19-d	$LD_{50} > 82$ µg product/bee or $> 4.2 \times 10^6$ CFU/bee
			Contact/15-d	$LD_{50} > 100$ µg product/bee or $> 5.1 \times 10^6$ CFU/bee
Apis mellifera	Pome fruits, solanaceous fruits, ornamentals	Thuricide SC (Btk SA-12)	Oral/4-d	$LD_{50} > 1 \times 10^9$ CFU/L
			Contact/5-d	$LD_{50} > 1 \times 10^9$ CFU/L
Field test				
-	-	-	-	-

Effects on terrestrial arthropods other than bees)

Species	Stage	Test Substance	Dose	Toxicity, infectivity and pathogenicity (endpoint, value or other description of

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				effects)
Laboratory Tests				
Aphidius rhopalosiphi	Adult	CoStar WG	Acute laboratory (glass plate), 12 kg product/ha	EC_{50} > EC_{50} > 12 kg product/ha corresponding to 1.1×10^{12} IU/ha
Typhlodromus pyri	Protonymphs	CoStar WG	Acute laboratory (glass plate), 12 kg product/ha	EC_{50} > 12 kg product/ha corresponding to 1.1×10^{12} IU/ha

Effects on other terrestrial invertebrates

Toxicity, infectivity and pathogenicity: (endpoint, value or other description of effects)	Due to available data on a closely related strain and available knowledge about *Bacillus thuringiensis* subsp. *kurstaki* in general it can be concluded that Btk SA-12 is not toxic, pathogenic or infective to earthworms.
Further information:	-

Effects on soil microorganisms

Due to available data on a closely related strain and available knowledge about *Bacillus thuringiensis* subsp. *kurstaki* in general it can be concluded that Btk SA-12 is not toxic, pathogenic or infective to soil micro-organisms.

Additional studies

None