Renewal
Assessment Report

Bacillus thuringiensis subsp. *aizawai* strain GC-91

Volume 1

June 2018

Rapporteur Member State: The Netherlands

Co-Rapporteur Member State: Germany

Version history

When	What
June 2018	Initial RAR (Amended March 2019)

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Level 1

Bacillus thuringiensis subsp. aizawai strain GC-91

1 Statement of subject matter and purpose for which this report has been prepared and background information on the application

1.1 Context in which the renewal assessment report was prepared

1.1.1 Purpose for which the renewal assessment report was prepared

The dossier for *Bacillus thuringiensis subsp. aizawai strain GC-91* is submitted to support the renewal of approval of this micro-organism under Regulation 1107/2009/EC.

Also, this dossier contains data to support renewal of national authorizations of the formulations, i.e. plant protection products containing this active substance.

1.1.2 Arrangements between rapporteur Member State and co-rapporteur Member State

Not relevant.

1.1.3 EU Regulatory history for use in plant protection products

The following information about *Bacillus thuringiensis* ssp *aizawai* GC-91 is available:

- Review report: *Bacillus thuringiensis* ssp. *aizawai*, strain GC-91, SANCO/1538/08 – rev. 4 d.d.13 December 2013

It is mentioned on the commissions website the substance is fulfilling the criteria of Annex VI of Regulation (EC) 2229/2004: Criteria for clear indications of no harmful effects.

The substance has been included in Annex I of Directive 91/414/EC on 8 December 2008 (Directive 2008/113/EC).

Rapporteur member state of the first assessment for Annex I inclusion of Dir. 91/414/EC was Italy.

1.1.4 Evaluations carried out under other regulatory contexts

The active substance had been registered by US-EPA and by PMRA.

1.2 Applicant information

1.2.1 Name and address of applicant(s) for approval of the active substance

Mitsui AgriScience International SA/NV
Avenue de Tervueren 270
B-1150 Brussels
Belgium

Mitsui AgriScience International SA/NV is represented by:
GAB Consulting GmbH
Ottenbecker Damm 10
21684 Stade
Germany

Contact person Mitsui AgriScience International SA/NV:
[redacted]
Contact person GAB Consulting GmbH:
[redacted]

1.2.2 Producer or producers of the active substance

Please refer to volume 4, C.1.1 (confidential information).

1.2.3 Information relating to the collective provision of dossiers

Not relevant. This dossier is solely supported by Mitsui AgriScience International S.A./N.V. and no task force is involved.

1.3 Identity of the micro-organism

1.3.1 Name and species description, strain characterisation	
1.3.1.1 Composition of material used for manufacturing of the formulated product	
Confidential information, please refer to Volume 4	

1.3.1.2 1.3.1.2Accession number in culture collection	*Bacillus thuringiensis* ssp. *aizawai*, strain GC-91, is deposited in the National Collection of Type Cultures (NCTC), at the Health Protection Agency, Centre for Emergency Preparedness and Response, Porton Down, Salisbury, Wiltshire, SP4 0JG, UK (formerly Central Public Health Laboratory, Colindale Avenue, London NW9 5HT, UK) under the reference number NCTC 11821.
1.3.1.3 Scientific name and taxonomic grouping, i.e. family, genus, species, strain, serotype, pathovar or any other denomination relevant to the micro-organism	
Taxonomy	Section: Endospore-forming Gram positive Rods and Cocci Family: Bacillaceae Genus: *Bacillus* Species: *Bacillus thuringiensis* Subspecies : *aizawai* Serotype: H7 Strain: GC-91 First description: Burges et al. (1991) (US Patent) N.5,063,055.
Indigenous or non-indigenous	Indigenous (Strain GC-91 is described as a trans-conjugant of the Btk strain HD 191-A2 and the Bta strain HD 135-S4, both derived from indigenous wild type strains)
Wild type	Yes
Spontaneous or induced mutant*	No
Genetically modified according to Directive 2001/18/EC*	No
* All known differences between the modified micro-organism and the parent wild strain must be provided	
1.3.1.4 Test procedures and criteria used for identification	
For original approval a set of methods have been applied for characterisation of the strain including morphological and biochemical characterization, serotyping, plasmid profiling, activity spectrum, fatty acid analysis, DNA fingerprinting AFLP, cry toxin analysis. For renewal, the strain was sequenced and specific markers were developed and validated allowing an unequivocal identification of Bta GC-91.	

1.3.1.5	**Common name or alternative and superseded names and code names used during the development**	CGA-237218 or NCTC 11821 The strain is deposited in the National Collection of Type Cultures (NCTC), at the Health Protection Agency, Centre for Emergency Preparedness and Response, Porton Down, Salisbury, Wiltshire, SP4 0JG (formerly Central Public Health Laboratory, Colindale Avenue, London NW9 5HT) under the reference number NCTC 11821.
1.3.1.6	**Relationship to known pathogens**	There are no records of the relationship to known plant or animal or human pathogens.
1.3.1.7	**Method of manufacture (synthesis pathway) of the active substance**	Confidential information, please refer to Volume 4.
1.3.2	**Specification of the material used for manufacturing of formulated products**	Confidential information, please refer to Volume 4.
1.3.3	**Content of the micro-organism**	$6.2x10^{10}$ CFU/g to $7.9x10^{10}$ CFU/g
1.3.4	**Identity and content of impurities, additives, contaminating micro-organisms**	
1.3.4.1	Significant impurities	None.
1.3.4.2	Relevant impurities	None.
1.3.4.3	Additives	None.
1.3.4.4	Contaminating micro-organisms	Complies with SANCO/12116/2012 rev. 0
1.3.5	**Analytical profile of batches**	Please refer to Volume 4

1.4 Information on the plant protection product

1.4.1 Applicant	Name: Mitsui AgriScience International S.A./N.V. Address: Avenue de Tervueren 270 B-1150 Brussels Belgium Contact person: [redacted] Phone: [redacted] Fax Email
1.4.2 Producer of the plant protection product	The information is confidential and can be found in volume 4.
1.4.3 Current, former and proposed trade names and development code numbers	
Trade Name	Agree 50 WG and Agree 50 WP
Code Number	Not applicable
1.4.4 Detailed quantitative and qualitative information on the composition of the plant protection product	
1.4.4.1 Composition of the plant protection product	The information is confidential and can be found in volume 4
1.4.4.2 Information on the active substances	*Bacillus thuringiensis* subsp. *aizawai* strain GC-91
1.4.4.3 Information on safeners, synergists and co-formulants	The information is confidential and can be found in volume 4
1.4.5 Type and code of the plant protection product	See 1.4.3
1.4.6 Function	insecticide
1.4.7 Field of use envisaged	Agree 50 WG is used for the control of leaf consuming caterpillars in agriculture, horticulture, orcharding, viticulture, tree nursery crops and forestry.
1.4.8 Effects on harmful organisms	Agree 50 WG is non-systemic and poisons the caterpillars. During sporulation *B. thuringiensis* produces inclusion bodies which are composed of insecticidal crystal proteins (ICP, also called Cry proteins or δ-endotoxins). These Cry toxins are highly toxic to a wide variety of important agricultural and health related insect pests of the order Lepidoptera.

1.5 Detailed uses of the plant protection product

1.5.1 Details of representative uses

PPP (product name/code):	Agree 50 WG	Formulation type:	WG
Active Substance:	*Bacillus thuringiensis* subsp. *aizawai* GC-91	Conc. of a.s.:	500 g/kg or 25,000 IU/mg, min. 8.5×10^{12} CFU/kg, max. 3.3×10^{13} CFU/kg)
Applicant:	Mitsui AgriScience International SA/NV	professional use	☒
Zone(s):	EU	non professional use	☒

1	2	3	4	5	6	7	8	9	10	11	12	13
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)	**Application**			**Application rate**			**PHI** (days)	**Remarks:** e.g. g safener/synergist per ha
					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 as/ha IU/ha CFU/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		

1	2	3	4	5	6	7	8	9	10	11	12	13
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)	**Application**			**Application rate**			**PHI** (days)	**Remarks:** e.g. g safener/synergist per ha
					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 as/ha IU/ha CFU/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
1	EU	Pome fruits (apple, pear)	F	*Cydia pomonella*	Foliar spray	BBCH 53-99 (April-October)	a) 6 (7) b) 6 (7)	a) 2.0 b) 12.0	a) 1000 5×10^{10} IU/ha min $1,7*10^{13}$- max $6,6 \times 10^{13}$ CFU/ha b) 6000 3×10^{11} IU/ha Min $1,02 \times 10^{14}$ -max $3,96 \times 10^{14}$ CFU/kg	1000-1500	-	Maximum spray concentration (0.4 %)-400 g product/HL
2	EU	Grapes	F	*Lobesia botrana, Eupoecilia ambiguella*	Foliar spray	BBCH 53-99 (April-October)	a) 6 (7) b) 6 (7)	a) 2.0 b) 12.0	a) 1000 g/ha 5×10^{10}IU/ha min $1,7*10^{13}$- max 6.6×10^{13} CFU/ha b) 6000 3×10^{11}IU/ha Min $1,02 \times 10^{14}$ -max $3,96 \times 10^{14}$ CFU/kg	200-1200	-	Maximum spray concentration (0.4 %)-400 g product/HL -
3	EU	Tomato	G	*Tuta absoluta*	Foliar spray	BBCH 12-89 (all seasons, January-December)	a) 6 (7) b) 6 (7)	a) 2.0 b) 12.0	a) 1000 g/ha 5×10^{10} IU/ha min $1,7*10^{13}$- max $6.6\ 10^{13}$ CFU/ha b) 6000 3×10^{11}IU/ha Min $1,02 \times 10^{14}$ -max $3,96 \times 10^{14}$ CFU/kg	500-1500	.	Maximum spray concentration (0.4 %)-400 g product/HL -
4	EU	Turf, Sports	F	*Spodoptera* spp.	Foliar spray	BBCH 12-89 (all seasons, January-December)	a) 6 (7) b) 6 (7)	a) 2.0 b) 12.0	a) 1000 g/ha 5×10^{10} IU/ha min $1,7*10^{13}$- max 6.6×10^{13} CFU/ha b) 6000 3×10^{11} IU/ha Min $1,02 \times 10^{14}$ -max $3,96 \times 10^{14}$ CFU/kg	1000-1500	.	-

1.5.2 Further information on representative uses

Products based on *Bacillus thuringiensis subsp. aizawai* are already authorised, and have been evaluated according uniform principles in the past. No undesirable or unintended side-effects have been observed. No necessary waiting period or other precautions are needed to avoid phytotoxic effects on succeeding crops.

1.5.3 Details of other uses applied for to support the setting of MRLs for uses beyond the representative uses

Not applicable.

1.5.4 Overview on authorisations in EU Member States

A multitude of products containing *Bacillus thuringiensis* is registered Europe-wide for the control of Lepidopteran larvae in various agricultural and horticultural crops, orchards, and forests.

Agree 50 WG has been approved in the EU for many years. A summary of current registrations of Agree WG (= Turex WG) and the similar product Turex WP, which are both based on *Bacillus thuringiensis* subsp. *aizawai* GC-91, can be found in the following table. This is not an exhaustive list.

Product / Code	Crop F/G	Country	Registration number	Product application rate per treatment (max)	Active substance application rate per treatment (max)	Number of treatments per season/ crop	Active substance total dose/ha (max)
Turex WG, Turex WP	Apple, pear, cherry, plum F	Denmark	738-1 738-2	2.0 kg/ha	1 kg/ha	3	3 kg/ha
Turex WG, Turex WP	Flowering cabbages, head cabbages and leafy cabbages F	Denmark	738-1 738-2	1.0 kg/ha	0.5 kg/ha	3	1.5 kg/ha
Turex WG, Turex WP	Tomato, cucumber and pepper plants G	Denmark	738-1 738-2	1.0 kg/ha	0.5 kg/ha	6	3 kg/ha
Turex WG, Turex WP	Ornamentals and nursery plants F, G	Denmark	738-1 738-2	1.0 kg/ha	0.5 kg/ha	6	3 kg/ha
Turex 50 WP	Flowering cabbages, head cabbages and leafy cabbages, Chinese mustard, kohlrabi F, G	Sweden	4492	1.0 kg/ha	0.5 kg/ha	3	1.5 kg/ha
Turex 50 WP	Tomato, cucumber, pepper, chili, aubergine, squash, melon G	Sweden	4492	1.0 kg/ha	0.5 kg/ha	6	3.0 kg/ha
Turex 50 WP	Lettuce F	Sweden	4492	1.0 kg/ha	0.5 kg/ha	3	1.5 kg/ha
Turex 50 WP	Ornamentals and nursery plants F, G	Sweden	4492	1.0 kg/ha	0.5 kg/ha	6	3.0 kg/ha
Turex 50 WP	Apple, pear, plum, cherry F	Sweden	4492	2.0 kg/ha	1.7×10^{13} CFU/ha	3	5.1×10^{13} CFU/ha
Turex 50 WP	Currants F	Sweden	4492	1.0 kg/ha	0.5 kg/ha	3	1.5 kg/ha
Turex 50 WP	Strawberry F, G	Sweden	4492	1.0 kg/ha	0.5 kg/ha	3	1.5 kg/ha
Turex 50 WP	Seed production in pine tree and spruce	Sweden	4492	2.0 kg/ha	1.0 kg/ha	3	3.0 kg/ha

Product / Code	Crop F/G	Country	Registration number	Product application rate per treatment (max)	Active substance application rate per treatment (max)	Number of treatments per season/ crop	Active substance total dose/ha (max)
	F, G						
Turex 50 WP	Flowering cabbages, head cabbages and leafy cabbages, broccoli, kohlrabi, turnip, swede F	Finland	1735	1.0 kg/ha	0.5 kg/ha	3	1.5 kg/ha
Turex 50 WP	Tomato, cucumber, pepper, chili G	Finland	1735	1.0 kg/ha	0.5 kg/ha	6	3.0 kg/ha
Turex 50 WP	Ornamentals and nursery plants F, G	Finland	1735	1.0 kg/ha	0.5 kg/ha	6	3.0 kg/ha
Turex 50 WP	Apple, pear, cherry, plum F	Finland	1735	2.0 kg/ha	1.0 kg/ha	3	3.0 kg/ha
Turex 50 WP	Currants F	Finland	1735	1.0 kg/ha	0.5 kg/ha	3	1.5 kg/ha
Turex 50 WP	Strawberry F, G	Finland	1735	1.0 kg/ha	0.5 kg/ha	3	1.5 kg/ha
Turex 50 WP	Seed production in pine tree and spruce F, G	Finland	1735	2.0 kg/ha	1.0 kg/ha	3	3.0 kg/ha
Turex 50 WP	Aubergine, squash, melon G	Finland	1735	1.0 kg/ha	0.5 kg/ha	6	3.0 kg/ha
Turex 50 WP	Lettuce, herbs F	Finland	1735	1.0 kg/ha	0.5 kg/ha	3	1.5 kg/ha
Turex 50 WP	Beetroot, radish, celery, celeriac, carrot, onion, garlic, leek, shallots	Finland	1735	1.0 kg/ha	0.5 kg/ha	3	1.5 kg/ha
Turex 50 WP	Beans	Finland	1735	1.0 kg/ha	0.5 kg/ha	3	1.5 kg/ha
Agree 50 WG	Root and tuber vegetables F, G	Germany	007638	1 kg/ha	0.5 kg/ha	3	1.5 kg/ha
Agree 50	Ornamentals	Germany	007638	1 kg/ha	0.5 kg/ha	6	3.0 kg/ha

Product / Code	Crop F/G	Country	Registration number	Product application rate per treatment (max)	Active substance application rate per treatment (max)	Number of treatments per season/ crop	Active substance total dose/ha (max)
WG	F, G						
Turex WG	Beetroot F, G	Belgium	10461P/B	1 kg/ha	0.5 kg/ha	3	1.5 kg/ha
Turex WG	Radish, black radish and radish rave F, G	Belgium	10461P/B	1 kg/ha	0.5 kg/ha	3	1.5 kg/ha
Turex WG	Carrots F, G	Belgium	10461P/B	1 kg/ha	0.5 kg/ha	3	1.5 kg/ha
Turex WG	Ornamentals F, G	Belgium	10461P/B	0.1 kg/hL (1 kg/ha if 1000 L water/ha)	0.5 kg/ha	6	3 kg/ha
Turex WG	Root and tuber vegetables F, G	Netherlands	15039 N	1 kg/ha	0.5 kg/ha	F: 3 per 12 months P: 6 per 12 months (3 times per growing cycle and 2 cycles per 12 months)	1.5 kg/ha
Turex WG	Ornamentals G	Netherlands	15039 N	1 kg/ha	0.5 kg/ha	36 per 12 months (6 times per growing cycle and 6 cycles per 12 months)	3 kg/ha per cycle
Turex WG	Public green F	Netherlands	15039 N	1 kg/ha	0.5 kg/ha	3 per 12 months	1.5 kg/ha
Turex spuitpoeder	Chicory F	Netherlands	11702 N	1 kg/ha	0.5 kg/ha	3	1.5 kg/ha
Turex spuitpoeder	Strawberry F, G	Netherlands	11702 N	1 kg/ha	0.5 kg/ha	3 per 12 months	1.5 kg/ha
Turex spuitpoeder	Berries (currants, gooseberry, blueberry, cranberry, mulberry, rose hips, kiwiberry, elderberry, other berries) F, G	Netherlands	11702 N	1 kg/ha	0.5 kg/ha	3 per 12 months	1.5 kg/ha
Turex spuitpoeder	Grapes F, G	Netherlands	11702 N	1 kg/ha	0.5 kg/ha	3 per 12 months	1.5 kg/ha

Product / Code	Crop F/G	Country	Registration number	Product application rate per treatment (max)	Active substance application rate per treatment (max)	Number of treatments per season/ crop	Active substance total dose/ha (max)
Turex spuitpoeder	Blackberry and raspberry family F, G	Netherlands	11702 N	1 kg/ha	0.5 kg/ha	3 per 12 months	1.5 kg/ha
Turex spuitpoeder	Lettuce F, G	Netherlands	11702 N	1 kg/ha	0.5 kg/ha	3 per 12 months	1.5 kg/ha
Turex spuitpoeder	Endive F, G	Netherlands	11702 N	1 kg/ha	0.5 kg/ha	3 per 12 months	1.5 kg/ha
Turex spuitpoeder	Spinach and similar F, G	Netherlands	11702 N	1 kg/ha	0.5 kg/ha	3 per 12 months	1.5 kg/ha
Turex spuitpoeder	Lamb's lettuce F, G	Netherlands	11702 N	1 kg/ha	0.5 kg/ha	3 per 12 months	1.5 kg/ha
Turex spuitpoeder	Bean (with pod) F, G	Netherlands	11702 N	1 kg/ha	0.5 kg/ha	3 per 12 months	1.5 kg/ha
Turex spuitpoeder	Fruiting vegetables of Cucurbitaceae (edible peel) F, G	Netherlands	11702 N	1 kg/ha	0.5 kg/ha	6 per 12 months	3.0 kg/ha
Turex spuitpoeder	Fruiting vegetables of Cucurbitaceae (non-edible peel) F, G	Netherlands	11702 N	1 kg/ha	0.5 kg/ha	6 per 12 months	3.0 kg/ha
Turex spuitpoeder	Fruiting vegetables of Solanaceae (tomato, sweet and chili pepper, eggplant) G	Netherlands	11702 N	1 kg/ha	0.5 kg/ha	6 per 12 months	3.0 kg/ha
Turex spuitpoeder	Cabbages (heading cabbages, cauliflower family, loose leaf cabbage family, kohlrabi) F, G	Netherlands	11702 N	1 kg/ha	0.5 kg/ha	3 per 12 months	1.5 kg/ha
Turex spuitpoeder	Radish family F, G	Netherlands	11702 N	1 kg/ha	0.5 kg/ha	3 per 12 months	1.5 kg/ha
Turex spu-	Carots	Netherlands	11702 N	1 kg/ha	0.5 kg/ha	3	1.5 kg/ha

Product / Code	Crop F/G	Country	Registration number	Product application rate per treatment (max)	Active substance application rate per treatment (max)	Number of treatments per season/ crop	Active substance total dose/ha (max)
itpoeder	F, G						
Turex spuitpoeder	Swede F	Netherlands	11702 N	1 kg/ha	0.5 kg/ha	3	1.5 kg/ha
Turex spuitpoeder	Beetroot F, G	Netherlands	11702 N	1 kg/ha	0.5 kg/ha	3	1.5 kg/ha
Turex spuitpoeder	Celeriac F, G	Netherlands	11702 N	1 kg/ha	0.5 kg/ha	3	1.5 kg/ha
Turex spuitpoeder	Onion and similar F	Netherlands	11702 N	1 kg/ha	0.5 kg/ha	3	1.5 kg/ha
Turex spuitpoeder	Stalk celery F	Netherlands	11702 N	1 kg/ha	0.5 kg/ha	3	1.5 kg/ha
Turex spuitpoeder	Leek F, G	Netherlands	11702 N	1 kg/ha	0.5 kg/ha	3	1.5 kg/ha
Turex spuitpoeder	Herbs (fresh use and dried) F, G	Netherlands	11702 N	1 kg/ha	0.5 kg/ha	3 per 12 months	1.5 kg/ha
Turex spuitpoeder	Floriculture F, G	Netherlands	11702 N	1 kg/ha	0.5 kg/ha	6 per 12 months	3.0 kg/ha
Turex spuitpoeder	Tree nursery crops F, G	Netherlands	11702 N	1 kg/ha	0.5 kg/ha	6 per 12 months	3.0 kg/ha
Turex spuitpoeder	Perennials F, G	Netherlands	11702 N	1 kg/ha	0.5 kg/ha	6 per 12 months	3.0 kg/ha
Turex spuitpoeder	Urban green	Netherlands	11702 N	1 kg/ha	0.5 kg/ha	3 per 12 months	1.5 kg/ha
Turex spuitpoeder	Forestry	Netherlands	11702 N	1 kg/ha	0.5 kg/ha	3 per 12 months	1.5 kg/ha
Agree 50 WG	Radish, red beet, swede F	United Kingdom	MAPP 17502	1 kg/ha	0.5 kg/ha	3	1.5 kg/ha
Agree 50 WG	Amenity vegetation, ornamental plant production	United Kingdom	MAPP 17502	1 kg/ha	0.5 kg/ha	6	3 kg/ha
AGREE WG	Grape F	Italy	14559	2 kg/ha	1 kg/ha	3	3 kg/ha
AGREE WG	Pome fruit (apple, pear) F	Italy	14559	2 kg/ha	1 kg/ha	3	3 kg/ha
AGREE	Stone fruit (peach, nectar-	Italy	14559	2 kg/ha	1 kg/ha	4	4 kg/ha

Product / Code	Crop F/G	Country	Registration number	Product application rate per treatment (max)	Active substance application rate per treatment (max)	Number of treatments per season/ crop	Active substance total dose/ha (max)
WG	ine, plum, cherry, apricot) F						
AGREE WG	Citrus F	Italy	14559	2 kg/ha	1 kg/ha	4	4 kg/ha
AGREE WG	Kiwifruit F	Italy	14559	2 kg/ha	1 kg/ha	4	4 kg/ha
AGREE WG	Olives F	Italy	14559	2 kg/ha	1 kg/ha	4	4 kg/ha
AGREE WG	Strawberry F, G	Italy	14559	2 kg/ha	1 kg/ha	4	4 kg/ha
AGREE WG	Cabbage F, G	Italy	14559	2 kg/ha	1 kg/ha	4	4 kg/ha
AGREE WG	Turnip, radish F, G	Italy	14559	2 kg/ha	1 kg/ha	4	4 kg/ha
AGREE WG	Tomato, sweet pepper, egg-plant F, G	Italy	14559	2 kg/ha	1 kg/ha	4	4 kg/ha
AGREE WG	Basil, chard F, G	Italy	14559	2 kg/ha	1 kg/ha	4	4 kg/ha
AGREE WG	Cucurbits F, G	Italy	14559	2 kg/ha	1 kg/ha	4	4 kg/ha
AGREE WG	Leafy vegetables (Lettuce and curled lettuce, spinach, leaf beet, teasel, celery, fennel, parsley, chives, fresh herbs) F, G	Italy	14559	2 kg/ha	1 kg/ha	4	4 kg/ha
AGREE WG	Bean, French bean, artichoke, rape, cole F, G	Italy	14559	2 kg/ha	1 kg/ha	4	4 kg/ha
AGREE WG	Potato F, G	Italy	14559	2 kg/ha	1 kg/ha	4	4 kg/ha
AGREE WG	Maize F	Italy	14559	2 kg/ha	1 kg/ha	Not stated	-
AGREE	Beet	Italy	14559	2 kg/ha	1 kg/ha	Not stated	-

Product / Code	Crop F/G	Country	Registration number	Product application rate per treatment (max)	Active substance application rate per treatment (max)	Number of treatments per season/ crop	Active substance total dose/ha (max)
WG	F						
AGREE WG	Tobacco F	Italy	14559	2 kg/ha	1 kg/ha	Not stated	-
AGREE WG	Cotton F	Italy	14559	2 kg/ha	1 kg/ha	Not stated	-
AGREE WG	Flower, ornamentals F	Italy	14559	2 g/hL	1 g/hL	Not stated	-
AGREE WG	Forest, poplar F	Italy	14559	2 kg/ha	1 kg/ha	Not stated	-
AGREE WG	Turf, sports F	Italy	14559	2 kg/ha	1 kg/ha	Not stated	-
AGREE WG	Urban green	Italy	14559	2 kg/ha	1 kg/ha	Not stated	-
AGREE	Grapes F	Italy	9477	2 kg/ha	1 kg/ha	3	3 kg/ha
AGREE	Pome fruit (apple, pear) F	Italy	9477	2 kg/ha	1 kg/ha	3	3 kg/ha
AGREE	Stone fruit (peach, nectarine, plum, cherry, apricot) F	Italy	9477	2 kg/ha	1 kg/ha	3	3 kg/ha
AGREE	Citrus F	Italy	9477	2 kg/ha	1 kg/ha	3	3 kg/ha
AGREE	Kiwifruit F	Italy	9477	2 kg/ha	1 kg/ha	3	3 kg/ha
AGREE	Olive tree F	Italy	9477	2 kg/ha	1 kg/ha	3	3 kg/ha
AGREE	Strawberry F, G	Italy	9477	2 kg/ha	1 kg/ha	4	4 kg/ha
AGREE	Cabbage F, G	Italy	9477	2 kg/ha	1 kg/ha	4	4 kg/ha
AGREE	Turnip, radish F, G	Italy	9477	2 kg/ha	1 kg/ha	4	4 kg/ha
AGREE	Tomato, sweet pepper, egg-plant F, G	Italy	9477	2 kg/ha	1 kg/ha	4	4 kg/ha
AGREE	Basil, chard	Italy	9477	2 kg/ha	1 kg/ha	4	4 kg/ha

Product / Code	Crop F/G	Country	Registration number	Product application rate per treatment (max)	Active substance application rate per treatment (max)	Number of treatments per season/ crop	Active substance total dose/ha (max)
	F, G						
AGREE	Cucurbits (water melon, melon, courgette, pumpkin, cucumber) F, G	Italy	9477	2 kg/ha	1 kg/ha	4	4 kg/ha
AGREE	Leafy vegetables (Lettuce and curled lettuce, spinach, leaf beet, teasel, celery, fennel, parsley, chives, fresh herbs) F, G	Italy	9477	2 kg/ha	1 kg/ha	4	4 kg/ha
AGREE	Bean, French bean, artichoke, rape, cole F, G	Italy	9477	2 kg/ha	1 kg/ha	4	4 kg/ha
AGREE	Potato F, G	Italy	9477	2 kg/ha	1 kg/ha	4	4 kg/ha
AGREE	Maize F	Italy	9477	2 kg/ha	1 kg/ha	Not stated	-
AGREE	Beet F	Italy	9477	2 kg/ha	1 kg/ha	Not stated	-
AGREE	Tobacco F	Italy	9477	2 kg/ha	1 kg/ha	Not stated	-
AGREE	Cotton F	Italy	9477	2 kg/ha	1 kg/ha	Not stated	-
AGREE	Flower, ornamentals F	Italy	9477	2 kg/ha	1 kg/ha	Not stated	-
AGREE	Forest, poplar F	Italy	9477	2 kg/ha	1 kg/ha	Not stated	-
AGREE	Turf, sports F	Italy	9477	2 kg/ha	1 kg/ha	Not stated	-
AGREE	Urban green	Italy	9477	2 kg/ha	1 kg/ha	Not stated	-
TUREX	Grape	Italy	11044	2 kg/ha	1 kg/ha	3	3 kg/ha
TUREX	Pome fruit (apple, pear)	Italy	11044	2 kg/ha	1 kg/ha	3	3 kg/ha

Product / Code	Crop F/G	Country	Registration number	Product application rate per treatment (max)	Active substance application rate per treatment (max)	Number of treatments per season/ crop	Active substance total dose/ha (max)
TUREX	Stone fruit (peach, nectarine, plum, cherry, apricot)	Italy	11044	2 kg/ha	1 kg/ha	4	4 kg/ha
TUREX	Citrus	Italy	11044	2 kg/ha	1 kg/ha	4	4 kg/ha
TUREX	Kiwifruit	Italy	11044	2 kg/ha	1 kg/ha	4	4 kg/ha
TUREX	Olive tree	Italy	11044	2 kg/ha	1 kg/ha	4	4 kg/ha
TUREX	Strawberry	Italy	11044	2 kg/ha	1 kg/ha	4	4 kg/ha
TUREX	Cabbage	Italy	11044	2 kg/ha	1 kg/ha	4	4 kg/ha
TUREX	Turnip cabbage, radish	Italy	11044	2 kg/ha	1 kg/ha	4	4 kg/ha
TUREX	Tomato, sweet pepper, eggplant	Italy	11044	2 kg/ha	1 kg/ha	4	4 kg/ha
TUREX	Cucurbitaceae (water melon, melon, courgette, pumpkin, cucumber)	Italy	11044	2 kg/ha	1 kg/ha	4	4 kg/ha
TUREX	Leafy vegetables (Lettuce and curled lettuce, spinach, leaf beet, teasel, celery, fennel, parsley, chives, fresh herbs	Italy	11044	2 kg/ha	1 kg/ha	4	4 kg/ha
TUREX	Beans, wax bean, artichoke, rape, turnip rape	Italy	11044	2 kg/ha	1 kg/ha	4	4 kg/ha
TUREX	Potato	Italy	11044	2 kg/ha	1 kg/ha	4	4 kg/ha
TUREX	Corn	Italy	11044	2 kg/ha	1 kg/ha	4	4 kg/ha
TUREX	Red beet	Italy	11044	2 kg/ha	1 kg/ha	4	4 kg/ha
TUREX	Tobacco	Italy	11044	2 kg/ha	1 kg/ha	4	4 kg/ha
TUREX	Cotton	Italy	11044	2 kg/ha	1 kg/ha	4	4 kg/ha
TUREX	Flower, ornamentals**	Italy	11044	2 kg/ha	1 kg/ha	4	4 kg/ha
TUREX	Forest, poplar	Italy	11044	2 kg/ha	1 kg/ha	4	4 kg/ha
TUREX	Turf, sports	Italy	11044	2 kg/ha	1 kg/ha	4	4 kg/ha
TUREX	Urban green	Italy	11044	2 kg/ha	1 kg/ha	4	4 kg/ha
DESIGN	Vine	Italy	15364	2 kg/ha	1 kg/ha	3	3 kg/ha

Product / Code	Crop F/G	Country	Registration number	Product application rate per treatment (max)	Active substance application rate per treatment (max)	Number of treatments per season/ crop	Active substance total dose/ha (max)
WG							
DESIGN WG	Pome fruit (apple, pear)	Italy	15364	2 kg/ha	1 kg/ha	3	3 kg/ha
DESIGN WG	Stone fruit (peach, nectarine, plum, cherry, apricot)	Italy	15364	2 kg/ha	1 kg/ha	4	4 kg/ha
DESIGN WG	Citrus	Italy	15364	2 kg/ha	1 kg/ha	4	4 kg/ha
DESIGN WG	Kiwifruit	Italy	15364	2 kg/ha	1 kg/ha	4	4 kg/ha
DESIGN WG	Olive tree	Italy	15364	2 kg/ha	1 kg/ha	4	4 kg/ha
DESIGN WG	Strawberry	Italy	15364	2 kg/ha	1 kg/ha	4	4 kg/ha
DESIGN WG	Cabbage	Italy	15364	2 kg/ha	1 kg/ha	4	4 kg/ha
DESIGN WG	Turnip cabbage, radish	Italy	15364	2 kg/ha	1 kg/ha	4	4 kg/ha
DESIGN WG	Tomato, sweet pepper, eggplant	Italy	15364	2 kg/ha	1 kg/ha	4	4 kg/ha
DESIGN WG	Cucurbitaceae (water melon, melon, courgette, pumpkin, cucumber)	Italy	15364	2 kg/ha	1 kg/ha	4	4 kg/ha
DESIGN WG	Leafy vegetables (Lettuce and curled lettuce, spinach, leaf beet, teasel, celery, fennel, parsley, chives, fresh herbs	Italy	15364	2 kg/ha	1 kg/ha	4	4 kg/ha
DESIGN WG	Beans, wax bean, artichoke, rape, turnip rape	Italy	15364	2 kg/ha	1 kg/ha	4	4 kg/ha
DESIGN WG	Potato	Italy	15364	2 kg/ha	1 kg/ha	4	4 kg/ha
DESIGN WG	Corn	Italy	15364	2 kg/ha	1 kg/ha	4	4 kg/ha
DESIGN WG	Red beet	Italy	15364	2 kg/ha	1 kg/ha	4	4 kg/ha

Product / Code	Crop F/G	Country	Registration number	Product application rate per treatment (max)	Active substance application rate per treatment (max)	Number of treatments per season/ crop	Active substance total dose/ha (max)
DESIGN WG	Tobacco	Italy	15364	2 kg/ha	1 kg/ha	4	4 kg/ha
DESIGN WG	Cotton	Italy	15364	2 kg/ha	1 kg/ha	4	4 kg/ha
DESIGN WG	Flower, ornamentals	Italy	15364	2 g/hL	1 g/hL	4	40 g/ha*
DESIGN WG	Forest, poplar	Italy	15364	2 kg/ha	1 kg/ha	4	4 kg/ha
DESIGN WG	Turf, sports	Italy	15364	2 kg/ha	1 kg/ha	4	4 kg/ha
DESIGN WG	Urban green	Italy	15364	2 kg/ha	1 kg/ha	4	4 kg/ha
Turex**	Olive	Spain	19.430	2.8 kg/ha	0.5 kg/ha	Not stated	-
Turex**	Sweet pepper	Spain	19.430	2 kg/ha	0.5 kg/ha	Not stated	-
Turex**	Tomato	Spain	19.430	2 kg/ha	0.5 kg/ha	Not stated	-
Turex**	Table grapes, vine grapes	Spain	19.430	2 kg/ha	0.5 kg/ha	Not stated	-
Turex**	Brassica vegetables	Spain	19.430	2 kg/ha	0.5 kg/ha	2	1.0 kg/ha
Turex**	Lettuce	Spain	19.430	2 kg/ha	0.5 kg/ha	2	1.0 kg/ha
Agree WP	Apple, pear F	Greece	1661	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Peach, nectarine, plum tree, cherry, apricot F	Greece	1661	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Lemon, orange, tangerine, clementine F	Greece	1661	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Kiwi F	Greece	1661	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Olive tree F	Greece	1661	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Strawberry F, G	Greece	1661	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Cabbages, flowering brassica: cauliflower, broccoli, etc., Head brassica: Brus-	Greece	1661	2 kg/ha	1 kg/ha	6	6 kg/ha

Product / Code	Crop F/G	Country	Registration number	Product application rate per treatment (max)	Active substance application rate per treatment (max)	Number of treatments per season/ crop	Active substance total dose/ha (max)
	sels sprouts, head cabbage. Leafy brassicas: Chinese cabbage, kale F, G						
Agree WP	Turnip, radish F, G	Greece	1661	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Tomato, pepper, eggplant F, G	Greece	1661	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Tomato, eggplant F, G	Greece	1661	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Basil, chard, pepper, eggplant F, G	Greece	1661	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Water melon, melon, zucchini, pumpkin, cucumber F, G	Greece	1661	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Lettuce and similar, radicchio, rucola, valeriana, spinach, cardus, fennel, celery, parsley, dill, fresh herbs F, G	Greece	1661	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Bean, green bean, artichoke, cole seed (oil seed rape) F	Greece	1661	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Potato F	Greece	1661	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Corn F	Greece	1661	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Beetroot F	Greece	1661	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Tobacco	Greece	1661	2 kg/ha	1 kg/ha	6	6 kg/ha

Product / Code	Crop F/G	Country	Registration number	Product application rate per treatment (max)	Active substance application rate per treatment (max)	Number of treatments per season/ crop	Active substance total dose/ha (max)
	F						
Agree WP	Cotton F	Greece	1661	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	*Lysiantus* spp., tulip, *Fresia* spp., *Lilium* spp, carnation, gerbera, rose F, G	Greece	1661	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	*Pinus* spp, Fir-tree, *Populus* spp, F	Greece	1661	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Turf, sports F	Greece	1661	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Urban green F	Greece	1661	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Apple, pear F	Portugal	3234	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Peach, nectarine, plum tree, cherry, apricot F	Portugal	3234	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Lemon, orange, tangerine, clementine F	Portugal	3234	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Kiwi F	Portugal	3234	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Olive tree F	Portugal	3234	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Strawberry F, G	Portugal	3234	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Cabbages, flowering brassica: cauliflower, broccoli, etc., Head brassica: brussels sprouts, head cabbage. Leafy brassicas: Chinese	Portugal	3234	2 kg/ha	1 kg/ha	6	6 kg/ha

Product / Code	Crop F/G	Country	Registration number	Product application rate per treatment (max)	Active substance application rate per treatment (max)	Number of treatments per season/ crop	Active substance total dose/ha (max)
	cabbage, kale F, G						
Agree WP	Turnip, radish F, G	Portugal	3234	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Tomato, pepper, eggplant F, G	Portugal	3234	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Tomato, eggplant F, G	Portugal	3234	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Basil, chard, pepper, eggplant F, G	Portugal	3234	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Water melon, melon, zucchini, pumpkin, cucumber F, G	Portugal	3234	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Lettuce and similar, radicchio, rucola, valeriana, spinach, cardus, fennel, celery, parsley, dill, fresh herbs F, G	Portugal	3234	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Bean, green bean, artichoke, cole seed (oil seed rape) F	Portugal	3234	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Potato F	Portugal	3234	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Corn F	Portugal	3234	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Beetroot F	Portugal	3234	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Tobacco F	Portugal	3234	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Cotton F	Portugal	3234	2 kg/ha	1 kg/ha	6	6 kg/ha

Product / Code	Crop F/G	Country	Registration number	Product application rate per treatment (max)	Active substance application rate per treatment (max)	Number of treatments per season/ crop	Active substance total dose/ha (max)
Agree WP	*Lysiantus* spp., tulip, *Fresia* spp., *Lilium* spp, carnation, gerbera, rose F, G	Portugal	3234	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	*Pinus* spp, Fir-tree, *Populus* spp, F	Portugal	3234	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Turf, sports F	Portugal	3234	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Urban green F	Portugal	3234	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Apple, pear F	Cyprus	2189	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Peach, nectarine, plum tree, cherry, apricot F	Cyprus	2189	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Lemon, orange, tangerine, clementine F	Cyprus	2189	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Kiwi F	Cyprus	2189	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Olive tree F	Cyprus	2189	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Strawberry F, G	Cyprus	2189	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Cabbages, flowering brassica: cauliflower, broccoli, etc., Head brassica: brussels sprouts, head cabbage. Leafy brassicas: Chinese cabbage, kale F, G	Cyprus	2189	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Turnip, radish F, G	Cyprus	2189	2 kg/ha	1 kg/ha	6	6 kg/ha

Product / Code	Crop F/G	Country	Registration number	Product application rate per treatment (max)	Active substance application rate per treatment (max)	Number of treatments per season/ crop	Active substance total dose/ha (max)
Agree WP	Tomato, pepper, eggplant F, G	Cyprus	2189	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Tomato, eggplant F, G	Cyprus	2189	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Basil, chard, pepper, eggplant F, G	Cyprus	2189	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Water melon, melon, zucchini, pumpkin, cucumber F, G	Cyprus	2189	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Lettuce and similar, radicchio, rucola, valeriana, spinach, cardus, fennel, celery, parsley, dill, fresh herbs F, G	Cyprus	2189	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Bean, green bean, artichoke, cole seed (oil seed rape) F	Cyprus	2189	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Potato F	Cyprus	2189	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Corn F	Cyprus	2189	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Beetroot F	Cyprus	2189	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Tobacco F	Cyprus	2189	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Cotton F	Cyprus	2189	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	*Lysiantus* spp., tulip, *Fresia* spp., *Lilium* spp, carnation, gerbera, rose	Cyprus	2189	2 kg/ha	1 kg/ha	6	6 kg/ha

Product / Code	Crop F/G	Country	Registration number	Product application rate per treatment (max)	Active substance application rate per treatment (max)	Number of treatments per season/ crop	Active substance total dose/ha (max)
	F, G						
Agree WP	*Pinus* spp, Fir-tree, *Populus* spp, F	Cyprus	2189	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Turf, sports F	Cyprus	2189	2 kg/ha	1 kg/ha	6	6 kg/ha
Agree WP	Urban green F	Cyprus	2189	2 kg/ha	1 kg/ha	6	6 kg/ha

Level 2

Bacillus thuringiensis subsp. aizawai strain GC-91

2 Summary of active substance hazard and of product risk assessment

2.1 Identity

Bacillus thuringiensis subsp. *aizawai* GC-91 (in the following abbreviated as Bta GC-91) is a transconjugant strain originating from a Bta and a Bt subsp. *kurstaki* strain. Bta in general occurs ubiquitous in soils on plants as well as in infested insects. Bta 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 Bta is mainly attributed to spore bound insecticidal pro-toxins (Cry toxins) which are ingested by the target pests and activated under alkaline conditions in the midgut of the larvae.
The manufacturing process of Bta GC-91 has not been changed since original approval.

Analysis of five batches GC-91 showed that no microbial pathogens of toxicological concern for human and animal health were detected. Quality tests did not reveal the presence of toxigenic pathogens producing nameable amounts of metabolites of toxicological concern for human and animal health. MPCA does not contain any additives.

2.2 Biological properties

2.2.1 Summary of biological properties of the active substance

Bacillus thuringiensis including *Bacillus thuringiensis* subsp. *aizawai* have been used since decades for control of Lepidopteran pests in agricultural settings. Bt is considered the most successful insect pathogen and presently comprises ~ 2% of the worldwide insecticidal market. Bt as a species occurs naturally in a range of environmental compartments such as soils, plant surfaces and infected insects. Background populations of Bt in the environment were found in the range from 10^4 to 10^8 CFU/g in soil and 0 – 10^4 CFU/g on plants. The insecticidal activity of Bta is mainly attributed to spore bound insecticidal pro-proteins (cry toxins) which are ingested by the target pests (Lepidopteran larvae) and activated under alkaline conditions in the midgut of the larvae.
Bta acts highly specific against members of the insect family of Lepidoptera. Some are also active against Diptera or Coleoptera. Strain specific Cry protein pattern confirmed main action of Bta GC-91 against Lepidopteran pests.
The bacterium has poor colonization ability and is not a good competitor in the soil. Its survival is dependent on the presence and activity of other soil microorganisms and protection from degradation effects of sunlight. 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.
For Bta GC-91, the possibility of exchange of genetic material before and during production of the technical material/end-use product is very unlikely. For manufacturing of Bta GC-91 technical material, a culture maintenance program is applied to ensure that only genetically unchanged and pure subcultures of the mother culture are used for fermentation. If a lost/gain of plasmid(s) would occur, it would be immediately visible in the results of bioassays for biopotency, which are carried out routinely, and with each single batch. There are published reports available indicating that an exchange of genetic material with closely related species upon field application cannot be completely ruled out. However, genetic exchange under natural conditions in the field is unlikely as it requires not only germination and growth of the donor strain, which only occurs in target insects, but also a high density of actively growing recipients. Even under these conditions, genetic exchange events have been found to occur at very low rates in laboratory experiments.
It was demonstrated that Bta GC-91 can produce Cry1Ac, Cry1C, Cry1D and Cry2A insecticidal pro-

teins. Apart from the Cry proteins several other insecticidal proteins produced by Bt and contributing to their mode of action have been described as well (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. In conclusion, confirming information provided previously, there is no indication in the published literature that metabolites involved in insecticidal activity of Bta GC-91 pose a risk for human health or the environment.
It was confirmed that Bta GC-91 is not able to produce cereulide and the highly cytotoxic type of CytK (type 1).
The ability of commercial Bt strains to produce *B. cereus*-enterotoxins and possible consequences for consumers is discussed since first evaluation of Bta GC-91. However, based on available knowledge on Bt including Bta GC-91, 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. Actually, the strain was shown to have a low toxigenic potential. Safety levels proposed for *B. cereus* in food stuff cannot be applied to commercial Bt strains as they differ significantly from pathogenic *B. cereus* strains.
Bta GC-91 has been shown to be sensitive to a range of antibiotics commonly used in human and veterinary medicine.

2.2.2 Summary of physical, chemical and technical properties of the plant protection product

Agree 50 WG was not the representative formulation for original approval of Bta strain GC-91. Hence, no data have been submitted or evaluated for this formulation before.

The in-use concentrations for the product are 0.133 – 0.4%. Physical, chemical and technical properties as well as storage stability were determined for the plant protection product Agree 50 WG.

Persistent foaming and suspensibility has not been determined at appropriate concentrations to cover the in-use concentration range. All other studies have been performed in accordance with the current requirements and the results are deemed to be acceptable. The appearance of the product is that of a pale brown, water dispersible granules-based formulation with a fish meal odour. It is not explosive nor flammable, and it has no oxidising properties. In 1% aqueous solution, it has a pH value of 6.40. The data indicate that Agree 50 WG is stable when stored at 30°C for 18 weeks and 2 years at 20ºC in polyester.

Persistent foaming and suspensibility have to be determined at 1%, The other physical, chemical and technical characteristics of Agree 50 WG are acceptable for a water dispersible granules (WG) formulation.

2.3 Data on application and efficacy

2.3.1 Summary of effectiveness

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. It can therefore be concluded that the dose rates for the representative uses are realistic.

2.3.2 Summary of information on the development of resistance

Bacillus thuringiensis subsp. *aizawai* Strain GC-91 is a microbial disruptor of insect midgut membranes. As with any insecticide, too frequent use of one type of Bt strain or one type of Bt delta-endotoxin can result in resistance of the insect to the active ingredient. *Bacillus thuringiensis* is a biological non-persistent insecticide thus reducing the chances of resistance build up. No cross-resistance has been reported between chemical insecticides and Bt products (Sarnthoy et al., 1997: Smirle et al., 2003). Certain insect species have developed a resistance to particular Bt products caused by prolonged use resulting in a reduction in binding of specific Cry toxins to the gut membrane binding site. However, indications are that certain pest species are susceptible to more than one Cry toxin produced by different Bt subspecies. Therefore, resistance management strategy of altering applications of Bt products can prove effective.

In conclusion, Bt products like any other insecticide should be used in IRM (Insecticide Resistance Management) or IPM (Integrated Pest Management) programs and not used over and over as the only insecticide of choice. IRM and IPM cultural practices are commonly in place already.

While resistance to *Bacillus thuringiensis* does occur, it can be concluded that the proposed GAP for the representative uses is still realistic. Resistance management will have to be evaluated by member-states during product renewal or authorisation, as it can depend on local resistant populations, agricultural practices and other variables.

2.3.3 Summary of adverse effects on treated crops

The representative products have all been authorised at Member State level for > 10 years and have therefore been assessed in line with Uniform Principles. For the purpose of renewal of the active substance no new information is required.

2.3.4 Summary of observations on other undesirable or unintended side-effects

The representative products have all been authorised at Member State level for > 10 years and have therefore been assessed in line with Uniform Principles. For the purpose of renewal of the active substance no new information is required.

2.4 Further information

2.4.1 Summary of methods and precautions concerning handling, storage, transport or fire

Handling and storage precautions:

Keep away from food, drink and animal feeding stuffs. When using, do not eat, drink or smoke. Wash hands and face before eating, drinking or smoking. Do not breathe dust or spray. Avoid contact with skin and eyes. Wear suitable protective clothing, gloves, eye/face protection and respiratory protection. (minimal A2, P3 filter).

Store in a cool, dry and a well-ventilated place, secure area out of the reach of children and domestic animals. Do not store food, beverages or tobacco products in the storage area. Prevent eating, drinking, tobacco usage, and cosmetic application in areas where there is a potential for exposure to the material. Always wash thoroughly after handling, high humidity and temperatures over 30ºC decrease the activity of the product. Do not contaminate waters with this product or its container.

Transport:

Transport of Agree 50 WG does not require special precautions.

Procedures to minimize the generation of waste:

Remainder of spray shall be diluted and sprayed over already treated areas. Totally cleaned packages can be given to the waste disposal or recycling system.

Hazardous combustion products:

Not known at the indicated conditions of use.

2.4.2 Summary of procedures for destruction or decontamination

The disposal of product has to be performed in accordance with all applicable federal, state and local environmental regulations. Wastes resulting from the use of Agree 50 WG, i.e. residual water dispersions can be disposed of at an approved waste disposal facility. Remainder of spray can also be diluted and sprayed over already treated areas.
The same procedure is applicable to larger quantities, which may occur very rarely only
Totally cleaned packages can be given to the regular waste disposal.

2.4.3 Summary of emergency measures in case of an accident

Containment of spillages:
Wear chemical safety glasses with side shields or chemical goggles, rubber gloves, rubber boots, long-sleeved shirt, long pants, head covering, and a NIOSH-approved dust or pesticide respirator with dust prefilters. For small spills, sweep up, keeping dust to a minimum and place in an approved chemical container. Wash the spill with water containing a strong detergent, absorb with pet litter or other absorbent material, sweep up and place in a chemical container. Seal the container and handle in an approved manner. Flush the area with water to remove any residue. Do not allow wash water to decontaminate water supplies.
Decontamination of areas, vehicles and buildings:
Refer to the above statement on spillages.
Disposal of damaged packaging, adsorbents and other materials:
Refer to the above statement on spillages.
Protection of emergency workers:
Refer to the above statement on spillages.
First aid measures:
- Eye contact: Immediately wash eyes with a large amount of running water. Hold eyelids apart to rinse the entire surface of the eyes and lids. Do not apply any medicating agent except on the advice of a physician.
- Skin contact: Wash with plenty of soap and water, including hair and under fingernails. Do not apply any medicating agent except on the advice of a physician. Remove contaminated clothing and decontaminate prior to use.
- Inhalation: Move victim from contaminated area to fresh air. Apply artificial respiration if necessary.
- Ingestion: If victim is fully conscious, immediately give large amounts of water to drink and induce vomiting. Never give anything by mouth to an unconscious person.

2.5 Analytical methods

A method for the determination of the biopotency of Bta GC-91 based technical powders has been presented before. A validated method for CFU counts of Bta GC-91 in the formulated product Agree 50 WG and aqueous suspensions is available and strain specific markers were developed and validated to unequivocal identify Bta GC-91. Microbial contaminant screenings were carried out following standard microbiological methods which are considered validated as such.

Bta GC-91, as all other Bt strains currently registered at EU level, was proposed for inclusion into Annex IV of Regulation (EC) No 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 (see B.7). No specific MRL was fixed for the active substance under Reg. (EC) No 396/2005, according to Art. 18(1)(b) of that Regulation. Up till now *Bacillus thuringiensis* subsp. *aizawai* strain GC-91 is not included in Annex IV due to delay at EFSA. Moreover, the default MRL of 0.01 mg/kg is not applicable because agencies are not used to follow enforcement or maintenance procedures for micro-organisms. Furthermore, the evaluation of the renewal to still going on to disprove the EFSA opinion that *Bacillus thuringiensis* subsp. *aizawai* strain GC-91 and pathogenic *B. cereus* strain are comparable.

Therefore, methods for the determination and quantification of residues are currently not required. However, strain specific markers are available which can be used for monitoring of the strain upon field application.

Measurements of residues in the environment

Active micro organism

No specific methods of analysis for viable residues in the environment are provided. Such methods are considered not required.

Cry1Ab:

Soil: extraction with phosphate buffered saline Tween, quantification with commercial ELISA kit. LOQ 0.25 ng/mL. Fortification recovery and extraction efficiency tests were done indicating acceptable recoveries, mean recoveries 51, 75, and 70% for 3 different soils

Water: processing via lyophilization and filter centrifugation, quantification with ELISA. Method detection limit 2.1 ng/L. Recoveries of the method 59.4, 95.5 and 79.2% for three different water types, with a mean of 78%.

2.6 Impact on human and animal health

2.6.1 Effects having relevance to human and animal health arising from exposure to the micro-organism or to impurities, additives, contaminating micro-organisms contained in the material used for manufacturing of formulated products

Two studies with Bta GC-91, submitted for first approval, were considered acceptable. There was no evidence that Bta may cause acute oral toxicity, pathogenicity or infectivity in mammals.

Two studies on acute respiratory toxicity study in rats with Bta GC-91 technical, submitted for first approval, were considered acceptable. There is no evidence that Bta may cause acute respiratory toxicity, pathogenicity or infectivity in mammals.

In total, four studies on systemic toxicity of Bta GC-91 were submitted for first approval and are considered acceptable. Neither intravenous nor intraperitoneal exposure revealed any evidence that Bta GC-91 acts toxic or pathogenic in mammals. Only evidence of toxicity or pathogenicity to mice was observed following intraperitoneal administration at the dose level of $> 10^6$ CFU/animal. Moreover, three studies following subcutaneous application of Bta GC-91 revealed no mortalities.

No study on repeated inhalatory exposure is required, since the acute toxicity studies provided for first approval of Bta GC-91 did not show any toxicological effects on the strain.

Additionally, no health related reactions were observed in personnel working with Bta-derived products for several years, thus, there is no evidence that Bta GC-91 may cause serious health effects after repeated inhalatory exposure in mammals.

Genotoxicity

Standard assays are not appropriate for testing the mutagenicity and genotoxicity of microorganisms. Genotoxicity testing should be conducted only for specific metabolites. Thus, no studies using Bta GC-91 are submitted.

Cell culture

Bta GC-91 is not an intracellular replicating micro-organism. Thus, according to Regulation (EU) No 283/2013, cell culture studies are not required.

Short toxicity and pathogenicity

A 13-weeks study in rats, submitted for first approval of Bta GC-91, was considered acceptable. No indications of direct toxicity, infectivity or pathogenicity following 13-weeks exposure to 10^8 CFU per day to Bta GC-91 technical were observed.

Table 5.1-1: Summary results of the acute toxicological studies on *Bacillus thuringiensis* subsp. *aizawai* GC-91

Study type	Species	Test item	Dose level	Findings	NOAEL	Reference
Acute oral	Rat	Bta, CGA-237218 technical, FL 910331	5050 mg per kg b.w. 1.1×10^{10} CFU per kg b.w.	One of ten animals died	LD_{50} > 5050 mg per kg b.w.	OECD: IIM 5.3.2/01
Acute oral	Rat	Bta, CGA-237218 technical	9.4×10^8 CFU per kg b.w.	No adverse effect, no infectivity	$LD_{50} > 9.4 \times 10^8$ per kg b.w.	OECD: IIM 5.3.2/02
Acute intratracheal	Rat	Bta, CGA-237218 technical	3.76×10^8 CFU/kg b.w.	2 of 36 animals died, transient signs of toxicity	$LD_{50} > 3.76 \times 10^8$ per kg b.w.	OECD: IIM 5.3.3/01:
Acute inhalation	Rat	CGA-237218 WP FL-910986	0.526 and 3.16 mg/L, 5.6 and 37.7×10^6 CFU /L	No mortalities, transient clin. signs	LC_{50} > 3.16 mg/L 37.7×10^6 CFU /L	OECD: IIM 5.3.3/02:
Acute intraperitoneal	Mouse	Bta, CGA-237218 technical, 91-7288	1.16×10^6 CFU/ mouse	No mortalities	1.16×10^6 CFU per mouse	OECD: IIM 5.3.4/01
Acute intraperitoneal	Mouse	Bta, CGA-237218 technical 911445	2.55×10^6 CFU/ mouse	No toxicity, no infectivity	2.55×10^6 CFU per mouse	OECD: IIM 5.3.4/02

Study type	Species	Test item	Dose level	Findings	NOAEL	Reference
Acute intra-peritoneal	Mouse	Bta, CGA-237218 FL-901966 FL-910039 FL-910040 FL-910041 FL-910042	10^8, 10^7, 10^6 CFU/ animal	10^8 CFU/mouse: 82% mortality; 10^7 CFU/mouse: 10% mortality; 10^6 CFU/mouse: no mortality , no toxicity	$LD_{50} > 10^7$ CFU per mouse	OECD: IIM 5.3.4/03
Acute intra-venously	Rat	Bta, CGA-237218 Technical	7.6×10^7 CFU per rat	No infectivity, no toxicity	7.6×10^7 CFU per rat	OECD: IIM 5.3.4/04
Subcutaneous	Mouse	CGA-237218 technical FL 900815	3.8×10^6 CFU/animal	No mortalities extremely irri-tating	$LD_{50} > 3.8 \times 10^6$ CFU/animal	OECD: IIM 5.5.1/02
Subcutaneous	Mouse	CGA-237218 technical FL 900816	2.66×10^6 CFU/animal	No mortalities, slightly irritat-ing	$LD_{50} > 2.66 \times 10^6$ CFU/animal	OECD: IIM 5.5.1/03
Subcutaneous	Mouse	CGA-237218 technical FL 900814	1.08×10^6 CFU/animal	No mortalities, non irritating	$LD_{50} > 1.08 \times 10^6$ CFU/animal	IIM 5.5.1/04
90 days, oral	Rat	Bta, CGA-237218 technical	10^8 CFU per animal per day for 13 weeks	No adverse ef-fects	10^8 CFU per animal per day	OECD: IIM 5.3.7.1/01

2.6.2 Impact on human health arising from exposure to the micro-organims or to impurities, additives, contaminating micro-organisms contained in the material used for manufacturing of formulated products

The similar formulation Agree 50 WP was already evaluated as representative formulation for original approval of Bta GC-91. The product contains *Bacillus thuringiensis* subsp. *aizawai* GC-91 at 1000 g/kg (corresponding to max. 6.6×10^{13} CFU/kg). It is intended for use as insecticide against noctuid moths (*Spodoptera* spp.) as well as torricidae (*Cydia pomonella, Lobesia botrana, Eupoecilia ambiguella*) and gelechiodiae (*Tuta absoluta*) in turf sports, pome fruits, grapes, and solanaceous fruits, respectively, by professional and non-professional users.

Table 5.2-1 Acute toxicity studies on Agree WP (applicable to Agree WG)

Study type	Species	Test item	Dose level	Findings	Reference
Acute oral toxicity	Rat	CGA-237218 WP FL-910959	4000, 5050 or 5500 mg /kg bw $2.6 – 3.5 \times 10^{10}$ CFU Bta	Mortalities (2/10) at 5050 mg /kg bw Transient clinical signs No adverse effect at 4000 or 5500 mg/kg bw LD_{50} > 5050 mg/kg bw	OECD: IIIM 7.1.1/01
Acute oral toxicity	Rat	Agree FL-920303 (CGA-237218 WP)	5050 mg /kg bw 1.2×10^{11} CFU Bta	Mortality (1/10) Transient clinical signs LD_{50} > 5050 mg/kg bw	OECD: IIIM 7.1.1/02
Acute dermal toxicity	Rabbit	Agree (CGA-237218 WP) FL-911716	2020 mg/kg b.w 2.85×10^{9} CFU Bta	No adverse effect LD_{50} > 2020 mg/kg bw	OECD: IIIM 7.1.2/01
Acute inhalation	Rat	CGA-237218 WP FL-910986	5.78 mg/L corresponding to 4.2×10^{7} CFU Bta	Transient clinical signs LC_{50} > 5.78 mg/L 4.2×10^{7} CFU	OECD: IIIM 7.1.3/01
Acute inhalation	Rat	Agree FL-921616	0.651 mg/L corresponding to 3.4×10^{8} CFU Bta	Transient clinical signs LC_{50} > 0.651 mg/L 3.4×10^{8} CFU	OECD: IIIM 7.1.3/02
Dermal irritation	Rabbit	Agree (CGA-237218 WP) FL-911716	2020 mg/kg b.w 2.85×10^{9} CFU Bta	Non-irritating	OECD: IIIM 7.1.2/01
Eye irritation/ infectivity	Rabbit	CGA-237218 WP FL-910959	38.8 mg 2.4×10^{8} CFU Bta	Non-Irritating	OECD: IIIM 7.1.5/01
Skin sensitisation* (Magnusson/ Kligman)	Guinea pig	Agree 50 WP	0.1 mL / animal	Not sensitizing	OECD: IIIM 7.1.6/01; KMP 7.2.3/01

* Study not acceptable.

Two studies on acute oral toxicity with the WP formulation were considered acceptable to assess oral toxicity of Agree 50 WG. In both studies, the LD_{50} was found to be greater than 5050 mg/kg bw corresponding to 1.2×10^{11} CFU.

Two studies on acute inhalation toxicity with the formulation Agree 50 WP were considered acceptable to assess acute inhalation toxicity of Agree 50 WG. The LC_{50} was found to be greater than 5.78 mg/L corresponding to 4.2×10^{7} CFU Bta GC-91 per L

The dermal toxicity study showed no acute toxicity following dermal exposure. The skin irritation study revealed only slight signs of dermal irritation.

Re-evaluation of the previously submitted study with the formulated product Agree 50 WG revealed only slight conjunctival irritation that was completely reversible within 10 days. However, according to the Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures, Agree 50 WG does not warrant classification as being an eye irritant. No hazard statement is required.

The study on skin sensitisation according to Magnusson & Kligman method is not considered acceptable. According to Regulation (EC) No 283/2013 (footnote 1 to point 5.2.1 in Part B), the available methods for testing dermal sensitisation are not suitable for testing microorganisms as microorganisms do not penetrate the skin. Therefore, neither Bta GC-91 nor Agree 50 WG warrant classification on sensitisation.

2.6.3 Summary of product exposure and risk assessment

No adverse effects were obtained in any study on toxicity, pathogenicity, or infectiveness, hence, calculations on the health risk for operators become meaningless: no target organ exists and no dose-effect response (NOAEL) can be determined. Btk/Bta preparations including the Bta GC-91 preparation Agree 50 WG are considered safe for operators, bystanders and residents, and workers.

2.7 Residues in or on treated products, food and feed

2.7.1 Persistence and likelihood of multiplication in or on crops, feedstuffs or foodstuffs

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

2.7.2 Further information required

2.7.3 Non-viable residues

Non-viable residues do not pose a risk to humans or the environment. Crystal proteins, the other major component in commercial Bt preparations apart from spores, are not toxic to mammals as indicated in different publications. In addition, crystal proteins are very unstable when exposed to light.

2.7.4 Viable residues

A number of studies monitored the occurrence of Bt on food. The cited publications report findings on fresh food of strains of Bt that are used commercially. These results have to be considered with care. In all studies the methods of identification are molecular methods that are not suitable to unequivocally distinguish closely related strains within the group of *Bacillus* spp. Moreover, in all studies, the strains from commercially known products were used as reference and therefore biased results to a large extend. The EFSA BIOHAZ panel indicates that most cases of food-borne outbreaks caused by the *B. cereus* group have been associated with concentrations above 10^5 CFU/g and that the levels of *B. cereus* that can be considered as a risk for consumers might be also valid for *B. thuringiensis*. However, this approach is not justified as insecticidal Bt strains differ significantly from pathogenic *B. cereus* strains in the physiological requirements (less stress resistant spores, lower germination and

growth rates, less well growing at high temperature and under microaerobic conditions), ecology and environmental behaviour (highly adapted to their insect hosts) and their toxigenic potential (lower potential for surface attachment and less aggressive against human cell lines, production of lower amounts of enterotoxins in the lab).

2.7.5 Summary of residue behavior resulting

As *B. thuringiensis* and its secondary metabolites are not persistent in soil water and air, and spores are not persistent on crop, half-life less than 1 day, multiplication on crops is not expected. It is generally agreed that persistence of Bt populations on plant surfaces is low. Factors restricting field persistence are UV-mediated degradation of spores, rain fall and plant growth (dilution effects), lack of nutrients and low humidity. The levels of *B. thuringiensis* reported in food are very variable, in most cases below 10^3 CFU/g. Because the microorganism is not infective or pathogenic and secondary metabolites are not expected on the crops no consumer risk is expected and no PHI is necessary. It can be concluded that the risk for consumers due to possible exposure of Bta GC-91 is acceptable.

2.8 Fate and behaviour in the environment

In the natural environment under favourable conditions *Bacillus thuringiensis* (Bt) cells exist in an active vegetative state where growth and colony formation can occur. Once conditions become unsuitable for continued growth and survival, sporulation takes place where endospores and crystalline inclusions, or proteins, are formed and the vegetative cells lyse. The endospores exist in a cryptobiotic state, akin to suspended animation and are quite durable. The crystalline proteins are the source of the δ-endotoxins which are damaging to specific insect species. When crystal proteins are ingested by insects, alkaline conditions in the gut initiate breakdown of the proteins which releases the δ-endotoxins. These immediately begin to interfere with internal cell gut structure soon leading to a cessation of feeding and, when enough crystal proteins were ingested, to eventual starvation.

2.8.1 Summary of fate and behaviour in soil

Bt 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 Bt can persist for long, up to years, 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, see active substance part KMA 7.1.1/01 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.

It is generally agreed that persistence of Bt populations on plant surfaces is low. Factors restricting field persistence are UV-mediated degradation of spores, rain fall and plant growth (dilution effects), lack of nutrients and low humidity. Natural levels of Bt on plant surfaces range between 3 and nearly 1000 CFU/g or cm² (Smith & Couche, 1991; Ignoffo et al., 1974; Hostetter et al., 1975).

The ecology of Bt is still poorly understood. It can readily be recovered in spore form from a variety of environments, including soil, plants, and dead insects. For many years, Bt has been regarded solely as an insect pathogen. The belief being that it is present on leaves, which, when consumed by susceptible insects, causes death with multiplication and subsequent sporulation occurring in the cadaver; the progeny spores and crystals of Bt being then available for ingestion by further, susceptible lepidopteran larvae. Infected larvae would, however, be expected to become recycled in the soil, and it is unclear how they could subsequently recolonize the phylloplane. There are indications that leafs can be colonized from treated soils with Bt (Bizarri, 2008). Spores can reach the lower leaves by rain splash, but no other mechanisms have clearly indicated how Bt in the soil can colonize plants and so have the opportunity to exert its pathogenic nature. Furthermore, several studies following the fate of Bt introduced into soil indicate that steady rates of decline are observed. Although it has been found that spores of Bt can germinate in the rhizosphere of some plants and also in the guts of some soil invertebrates, little multiplication seems to occur. Spores of Bt can survive for a longer period in nature.

Endotoxins formed by Bt are degraded in soils. Several scientifically peer reviewed papers show that half-lives for the endotoxins range from a few days to a few weeks. The persistence of Cry proteins in soil is low. Biodegradation in soil is demonstrated. DT50'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 DT90's < 40 days for Cry3Bb1. Under anaerobic conditions degradation of the endotoxins is slower with half-lives ranging from 46 -141 days.

Predicted Environmental Soil Density (PEDs) of the product Agree 50 WG

Assumptions:
Application rate Agree 50 WG: 2 kg/ha (equivalent to 1000 g a.s./ha or 6.6×10^{13} CFU/ha based on max. content). Accumulated application rate (up to 6 treatments): 12 kg product/ha, equivalent to 12000 g a.s./ha or 3.96×10^{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. No instant growth and decline of Bt. According to the PED_{soil} calculation the expected initial density is 16.0 mg product/kg dry soil, corresponding to 5.28×10^8 CFU/kg dry soil.

2.8.2 Summary of fate and behaviour in water

Water is not the natural habitat of Bt germination of conidia and therefore multiplication in water is not expected, since Bt 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, Bt 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 Bta is a temporarily limited incidence only.

The persistence of Cry proteins water is low, though hydrolysis seems not a major degradation route (DT50 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.

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.

Predicted surface density (PEDsw) of the product Agree 50 WG

The envisaged field of use as a foliar treatment in 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 (orchard). For the results see **Table 2.8.2-01**.

2.8.3 Summary of fate and behaviour in air

From the information from the original evaluation of *B. thuringiensis* subsp. *aizawai* GC-91 a rapid degradation of Bta 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; see 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.

Table 2.8.2-01 Calculation of the predicted environmental density of Agree 50 WG and *B. thuringiensis* in lentic water bodies (PED_{sw}) after 6 applications at 2 kg Agree 50 WG/ha in pome fruits

	Application rate[a)]	**Relevant drift rate [%][b)]**	**Amount reaching the water**	**Water volume (30 cm water layer) [L/m2]**	**Initial PED_{SW}**
Agree 50 WG	12 kg/ha	9.21	110.52 mg/m²	300	368.4 µg/L
***Bacillus thuringiensis* subsp. *aizawai* GC-91**	6 kg/ha	9.21	55.26 mg/m²	300	184.2 µg/L
	3.96×10^{14} CFU/ha		3.65×10^{9} CFU/m²		**1.22×10^{7} CFU/L**
***Bacillus thuringiensis* subsp. *aizawai* GC-91**	6 kg/ha	9.21	55.26 mg/m²	210[c)]	263.1 µg/L
	3.96×10^{14} CFU/ha		3.65×10^{9} CFU/m²		**1.74×10^{7} CFU/L**

a) Accumulated application rate, assuming no degradation between applications
b) Drift value for 6 applications in fruit crops (late)
c) TOXSWA standard ditch recommended by the RMS

2.8.4 Summary of mobility

From the information from the original evaluation of Bt 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.

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 active substance part Vol. 3 – 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 in groundwater.

2.9 Effects on non-target species

2.9.1 Summary of effects on birds (and other terrestrial vertebrates)

The previous DAR included a risk assessment according to SANCO document 4145/2000. RMS would like to note that it was agreed in PRAPeR M2 that the guidance document SANCO/4145/2000 was intended for chemical substances and is considered less relevant for plant protection products containing micro-organisms. During PRAPeR M2 it was agreed that, with the lack of appropriate exposure scenario's for micro-organisms, a worst case risk assessment could be performed by comparing the amount of CFU applied, or present in the application liquid to the endpoint of the study.

The RMS included the following risk assessment for birds and mammals.

- **Birds**

The data provided an endpoint of > 3.53 x 10^{11} CFU/kg b.w./day
The density of spores in the WG formulation is 3.3 x10^{13} CFU/kg. The applicant claims a maximum concentration of 0.4% in the spraying liquid corresponding to 4 g product/L. Therefore the resulting the maximum concentration in the spray liquid is 1.32 x 10^{11} CFU/L.

The exposure via drinking water is considered relevant. According to the EFSA bird mammal guidance

document (EFSA Journal 2009; 7(12):1438), the worst-case for drinking water is a small granivorous bird with a body weight of 15.3 g, with a drinking rate of 7.0 mL/day, equivalent to 0.46 L/kg bw/d. Based on the worst PECsw of 1.22 x 10^7 CFU/L for the applications in pome fruit, the daily dose is 0.56 x 10^7 CFU/kg bw/d. This value is below the endpoint for birds and therefore the risk through drinking water is considered acceptable.

[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.

- **Mammals**

The acute oral LD_{50} of *Bta* technical was greater than 9.4 x 10^8 CFU/kg bw (refer to toxicology section). The test substance is not toxic, infective or pathogenic on the basis of the acute oral toxicity study in male and female rats.

The daily water intrake of a small granivorous mammal with a body weight of 21.7 g is 0.24 L/kg bw/d. Considering the PECsw of 1.22 x 10^7 CFU/L for the applications in pome fruit, the daily dose is 0.29 x 10^7 CFU/kg bw/d. This value is below the endpoint for mammals and therefore the risk through drinking water is considered acceptable.

2.9.2 Summary of effects on aquatic organisms

The maximum PECsw was calculated for applications in pome fruits and grapes and amounts 1.22 x 10^7 CFU/L. The trigger TER values were validated with data for chemicals. Therefore, RMS will use instead the concept of margin of safety

Species	**PECsw CFU/L**	**Endpoint CFU/L**	**MoS**
Cyprinodon variegatus	1.22 x 10^7	> 2.1 x 10^9	> 172
Daphnia magna	1.22 x 10^7	6.2 x 10^8	51
Daphnia magna	1.22 x 10^7	> 25.5 x 10^6	> 2.1
Palaemonetes vulgaris	1.22 x 10^7	1.9 x 10^9 (diet)	156 (diet)
Scenedesmus subspicatus	1.22 x 10^7	3.6 x 10^9	295

The MoS for exposure via water are above the unit for all organisms.

2.9.3 Summary of effects on bees

The applicant claims for pome fruits, grapes and tomato a maximum intended concentration of Agree WG in the spraying liquid of 0.4 % which corresponds to 4 g product/L. Considering the concentration of 25000 IU/mg product, this translates to 1 x 10^8 IU/L or 1 x 10^5 IU/mL. For the exposure in turf , a maximum product rate of 2 kg/ha is applied in a minimum of 1000 L/ha. Considering the concentration of 25000 IU/mg product, this translates to 5 x 10^7 IU/L or 5 x 10^4 IU/mL.

These field application rates are higher than the concentrations of the *Bta* containing product at which the toxicity was measured in the test with *Bombus terrestris* (bumble bee) by Mommaerts V. et al. (2009). No toxicity was found for the *Btk* containing product (highest test dose 1.6 x10^4 IU/mL).

The applicant had the following argumentation in order to refine the possible risk to bumblebees:

- *the endpoint is not strain specific (as Bta GC-91 is a transconjugant between Btk and Bta also the endpoint for Dipel could be used) It has to be noted also that both, the exposure level and also the exposure time in the study of Mommaerts et al (2009) represent unrealistic worst case conditions*
- *the concentration in the spraying liquid is the highest possible field exposure only occurring during spraying. Usually dilution factors are applied e.g. a factor of 5 is used for calculation of drinking water exposure for birds and mammals for sources such as puddles or axes of leaves. If such a factor is considered the exposure would decrease to 2 x 10^4 IU/mL already.*
- *the high concentration will not be maintained for long but the Bt spores and associated cry toxins are considered to disappear quickly under field conditions (recorded half-life times of spores and pro-toxins are in the range of some hours to days)*
- *the authors have been aware about the fact that the chosen exposure scenario represents unrealistic worst case conditions and concluded that in general Bt based products can be considered safe for bumble bees.*

As mentioned above, the Btk-based product Dipel did not show any side effects at the MFRC et al. As Bta GC-91 is a transconjugant strain between a Btk and a Bta, this result should be taken into consideration also.

The RMS agrees that the exposure in the study by Mommaerts V. et al. (2009) represents a worst-case scenario. Regarding the transconjugant, the RMS is of opinion that it is unknown whether the *Bta* and *Btk* strains used in this study contain the same plasmids as the two strains used for the production of the transconjugant strain *Bta* GC-91. As a result, the conclusion from this study is that there can be effects on bumblebees however, it is uncertain what the real effect of the transconjugant will be.

The authors did conclude that in general, the *Bt* strains are safe to bumblebees, but in some cases there were detrimental effects that depend on strain and route of exposure. The authors state that routine testing of lethal and sublethal effects is recommended to ascertain a safe combined use of Bt products and bumblebees in modern agriculture.

No effect on the honey bees were observed in the acute test with the *Btk* at concentrations higher than the current field application rates.

In the case of *Bta* GC-91, the 5 day LC_{50} is higher than the maximum intended field rate for all the crops. The 10 day LC_{50}, however is below the maximum intended field rate for all the crops. Taking these results into account it cannot be excluded that the transconjugant is chronically toxic to bees. The applicant argues that the spores and associated Cry proteins cannot be maintained in the field for a long time with recorded half-lives of hours to days. The RMS requests the applicant to provide data to support this claim.

In the study by Kleiner, R. (1992), the tested dose of 2 g a.s./L covers for the applications as per GAP. The applicant claims maximum spray concentration of 400 g product/ HL which is equivalent to 2 kg a.s./L for the uses in pome fruits, grape and tomato. For the uses in sport and turf the maximum application rate is 1 kg a.s./L. Considering these and that the LD_{50} was higher than the maximum tested dose of 2 g a.s./L, the product containing the transconjugant Bta GC-91 is not acutely toxic to honeybees.

To conclude, the additional data is required in order to assess if the potential risk to bumblebees seen in the study by Mommaerts et al. (2009) is relevant for the current transconjugant and if it is relevant in the field situations. Furthermore, additional data on the half-lives of the Cry proteins are required in order to exclude the chronic

toxicity to bees. Information on infectivity and pathogenicity to honey bees and bumble bees must be submitted. The RMS is of opinion that if no further information is provided a restriction sentence is necessary prohibiting the application of the product when the crop is flowering.

2.9.4 Summary of effects on arthropods other than bees

The toxicity of the plant protection product was investigated in the studies with *Aphidius rhopalosiphi* and *Typhlodromus pyri.* In the study with A. *rhopalosiphi* while there were no effects on mortality, significant effects on reproduction were recorded at the application rate of 4.5 kg product/ha. In case of *Typhlodromus pyri,* no effects on mortality and reproduction were recorded at the application rate of 4.5 kg product/ha.

According to the current GAP, the maximum rate per application is 2 kg product/ha with a max total rate per crop/season of 12 kg a.s./ha. According to the information provided in Volume 1, it is not expected that the spores will survive longer than 10 days (depending on the formulation) and 1 month on broccoli and celery leaves. Therefore, for the current uses it is not expected that an accumulation between applications will occur. Considering these, the tested dose of 4.5 kg/ha from the *Aphidius rhopalosiphi* and *Typhlodromus pyri* tests is considered sufficient to cover the applications as per GAP for the field and greenhouse applications.

Regarding the studies with the active substance, the results were expressed in CFU/g feed which does not allow for a direct comparison with the current application rates.

2.9.5 Summary of effects on earthworms and other soil non-target macro-organisms

The acute toxicity to earthworms was investigated in the test by Winkler, J. (1992a). under the conditions of this test there was no mortality to earthworms at concentrations of 500 and 1000 mg/kg d.w. concentrations which are 31 and 62 times, respectively higher than intial PED in soil. Therefore no acute toxicity to earthworms from the application of the current product is expected.

Earthworm immunity has extensively been studied and earthworms have served as an important experimental model for immunologic research. Earthworms have evolved effective innate defence mechanisms for survival in often hostile habitats, and have experienced a long time of co-evolution with micro-organisms in their environment (Bilej, 2010). Earthworms are capable to cope with soilborne micro-organisms without being infected or negatively affected due to the long-time evolutionary co-existence (SANCO/12117/2012)[2]. Only few studies indicated pathogenic effects to earthworms, however the observed pathogenic effects of *Bacillus thuringiensis* subsp. *thuringiensis* in a prolonged study with *Lumbricus terrestris* were not attributed to the tested mBCA (Smirnoff & Heimpel, 1961; cited in SANCO/12117/2012). Addison and Holmes (1996, cited in SANCO/12117/2012) observed detrimental impacts of Bt-formulations on earthworms and other non-target soil organisms, but found no effect of unformulated and aqueous Btk at 1000 times the field concentration.

[2] Working Document to the Environmental Safety Evaluation of Microbial Biocontrol Agents, SANCO/12117/2012-rev.0, September 2012, EUROPEAN COMMISSION HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL Directorate E – Safety of the food chain Unit E.3 – Chemicals, contaminants, pesticides.

2.9.6 Summary of effects on soil micro-organisms

The maximum tested dose of 26.2 mg product/kg soil dw was 1.6 times higher than the PEDsoil of 16 mg product/kg soil dw. The calculated application rate represents a worst-case as it represents an accumulated application rate.
Since there were no effects at the maximum application rate, it can be concluded that there is no risk to soil microflora from the application of the product.

2.9.7 Summary of effects on other non target (flora and fauna)

According to the information provided in the previous version of the DAR *Bacillus thuringiensis* spp. *aizawai*, is toxic specifically to insects of the Lepidoptera order and no effects on terrestrial plants from applications of Bta in insecticidal formulations targeted specifically at these insects is expected This is further envisaged considering results from studies on algae. In addition evidence from over fifty phytotoxicity trials performed on twelve different crops in the USA with Javelin Biological Insecticide and SAN 415I SC 353 support the lack of toxicity expected on terrestrial plants following application of Btk containing formulations in the fields. The highest mean toxicity was observed in trials (n = 3) with Bok choy (*Brassica chinensis*) at 4.3%, the next highest mean toxicity was in trials (n = 10) with Sugar beet (*Beta vulgaris*) at 1.7% (Anonymous, year unknown). These results may be extrapolated to Bta due to their family relationship.

2.9.8 Summary of effects on biological methods for sewage treatment

No additional data submitted. It is not expected that Bta will survive in the sewage treatment plant.

2.9.9 Summary of product exposure and risk assessment

Refer to the sections above.

2.10 Classification and labelling

2.10.1 Classification and Labelling of the active substance

No classification required. As a precautionary measure the sentence “This product does contain micro-organisms and may cause sensitisation by skin contact" should be included on the label.

2.10.2 Classification and Labelling of the plant protection product

No classification required. As a precautionary measure the sentence “This product does contain micro-organisms and may cause sensitisation by skin contact" should be included on the label.

2.11 Relevance of metabolites in groundwater

Not applicable for this MPCA.

2.12 Consideration of isomeric composition in the risk assessment

No information is required as micro-organisms do not have isomers.

2.13 Residue definitions

2.13.1 Definition of residues for exposure/risk assessment

No residue definition is required.

2.13.2 Definition of residues for monitoring

No residue definition is required.

Level 3

Bacillus thuringiensis subsp. aizawai strain GC-91

3 Proposed decision with respect to the application

3.1 Background to the proposed decision

3.1.1 Proposal on acceptability against the decision making criteria – Article 4 and annex II of regulation (EC) No 1107/2009

3.1.1.1 Article 4				
		Yes	No	
i)	It is considered that Article 4 of Regulation (EC) No 1107/2009 is complied with. Specifically the RMS considers that authorisation in at least one Member State is expected to be possible for at least one plant protection product containing the active substance for at least one of the representative uses.	x		
3.1.1.2 Submission of further information				
		Yes	No	
i)	It is considered that a complete dossier has been submitted	x		
ii)	It is considered that in the absence of a full dossier the active substance may be approved even though certain information is still to be submitted because: (a) the data requirements have been amended or refined after the submission of the dossier; or (b) the information is considered to be confirmatory in nature, as required to increase confidence in the decision.			*n.a.*
3.1.1.3 Restrictions on approval				
		Yes	No	
	It is considered that in line with Article 6 of Regulation (EC) No 1107/2009 approval should be subject to conditions and restrictions.		x	

3.1.1.4 Criteria for the approval of an active substance				
Dossier				
		Yes	No	
	It is considered the dossier contains the information needed to establish, where relevant, Acceptable Daily Intake (ADI), Acceptable Operator Exposure Level (AOEL) and Acute Reference Dose (ARfD).	X		No reference values are required.
	It is considered that the dossier contains the information necessary to carry out a risk assessment and for enforcement purposes (relevant for substances for which one or more representative uses includes use on feed or food crops or leads indirectly to residues in food or feed). In particular it is considered that the dossier: (a) permits any residue of concern to be defined; (b) reliably predicts the residues in food and feed, including succeeding crops (c) reliably predicts, where relevant, the corresponding residue level reflecting the effects of processing and/or mixing; (d) permits a maximum residue level to be defined and to be determined by appropriate methods in general use for the commodity and, where appropriate, for products of animal origin where the commodity or parts of it is fed to animals; (e) permits, where relevant, concentration or dilution factors due to processing and/or mixing to be defined.	X		No consumer risk assessment is required (see Volume 3 MA B.7)
	It is considered that the dossier submitted is sufficient to permit, where relevant, an estimate of the fate and distribution of the active substance in the environment, and its impact on non-target species.	X		
Efficacy				
		Yes	No	
	It is considered that it has been established for one or more representative uses that the plant protection product, consequent on application consistent with good plant protection practice and having regard to realistic conditions of use is sufficiently effective.	x		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. It can therefore be concluded that the dose rates

				for the representative uses are realistic.
Relevance of metabolites				
		Yes	No	
	It is considered that the documentation submitted is sufficient to permit the establishment of the toxicological, ecotoxicological or environmental relevance of metabolites.	X		Not relevant for MPCA.
Composition				
		Yes	No	
	It is considered that the specification defines the minimum degree of purity, the identity and maximum content of impurities and, where relevant, of isomers/diastereo-isomers and additives, and the content of impurities of toxicological, ecotoxicological or environmental concern within acceptable limits.			Not relevant for microorganisms.
	It is considered that the specification is in compliance with the relevant Food and Agriculture Organisation specification, where such specification exists.			Not relevant for microorganisms.
	It is considered for reasons of protection of human or animal health or the environment, stricter specifications than that provided for by the FAO specification should be adopted			Not relevant for microorganisms.
Methods of analysis				
		Yes	No	
	It is considered that the methods of analysis of the active substance, safener or synergist as manufactured and of determination of impurities of toxicological, ecotoxicological or environmental concern or which are present in quantities greater than 1 g/kg in the active substance, safener or synergist as manufactured, have been validated and shown to be sufficiently specific, correctly calibrated, accurate and precise.	X		Sufficient validated methods are available.
	It is considered that the methods of residue analysis for the active substance and relevant metabolites in plant, animal and environmental matrices and drinking water, as appropriate, shall have been validated and shown to be sufficiently sensitive with respect to the levels of concern.			No residue methods of analysis are required.
	It is confirmed that the evaluation has been carried out in accordance with the uniform principles for evaluation and authori-	X		

	sation of plant protection products referred to in Article 29(6) of Regulation 1107/2009.			
Impact on human health				
Impact on human health - ADI, AOEL, ARfD				
		Yes	No	
	It is confirmed that (where relevant) an ADI, AOEL and ARfD can be established with an appropriate safety margin of at least 100 taking into account the type and severity of effects and the vulnerability of specific groups of the population.	X		No reference values are required.
Impact on human health - proposed genotoxicity classification				
		Yes	No	
	It is considered that, on the basis of assessment of higher tier genotoxicity testing carried out in accordance with the data requirements and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as mutagen category 1A or 1B.**			Not relevant for microorganisms.
Impact on human health - proposed carcinogenicity classification				
		Yes	No	
i)	It is considered that, on the basis of assessment of the carcinogenicity testing carried out in accordance with the data requirements for the active substances, safener or synergist and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as carcinogen category 1A or 1B.**			Not relevant for microorganisms.
ii)	Linked to above classification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation			n.a.

	(EC) No 396/2005.			
Impact on human health – proposed reproductive toxicity classification				
		Yes	No	
i)	It is considered that, on the basis of assessment of the reproductive toxicity testing carried out in accordance with the data requirements for the active substances, safeners or synergists and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as toxic for reproduction category 1A or 1B.**			Not relevant for microorganisms.
ii)	Linked to above classification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			n.a.
Impact on human health - proposed endocrine disrupting properties classification				
		Yes	No	
i)	It is considered that **the substance SHOULD BE classified or proposed for classification** in accordance with the provisions of Regulation (EC) No 1272/2008, as **carcinogenic category 2 and toxic for reproduction category 2 and on that basis shall be considered to have endocrine disrupting properties**			Not relevant for microorganisms
ii)	It is considered that **the substance SHOULD BE classified or proposed for classification** in accordance with the provisions of Regulation (EC) No 1272/2008, as **toxic for reproduction category 2 and** in addition the RMS considers the substance **has toxic effects on the endocrine organs and on that basis shall be considered to have endocrine disrupting properties**			Not relevant for microorganisms
iii)	Linked to either i) or ii) immediately above. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic			n.a.

	proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			
Fate and behaviour in the environment				
Persistent organic pollutant (POP)				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a persistent organic pollutant (POP) as laid out in Regulation 1107/2009 Annex II Section 3.7.1.		X	Bt are considered not to be persistent these micro-organisms are naturally present in soil
Persistent, bioaccumulative and toxic substance (PBT)				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a persistent, bioaccumulative and toxic (PBT) substance as laid out in Regulation 1107/2009 Annex II Section 3.7.2.		X	Bt are considered not to be persistent these micro-organisms are naturally present in soil.
Very persistent and very bioaccumulative substance (vPvB)				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a a very persistent and very bioaccumulative substance (vPvB) as laid out in Regulation 1107/2009 Annex II Section 3.7.3.		X	Bt are considered not to be persistent these micro-organisms are naturally present in soil.
Ecotoxicology				
		Yes	No	
	It is considered that the risk assessment demonstrates risks to be acceptable in accordance with the criteria laid down in the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) under realistic proposed conditions of use of a plant protection product containing the active substance, safener or synergist. The RMS is content that the assessment takes into account the severity of effects, the uncertainty of the data, and the number of organism groups which the active substance, safener or synergist is expected to affect adversely by the intended use.		x	The additional data is required in order to assess if the potential risk to bumblebees seen in the study by Mommaerts et al. (2009) is relevant for the current transconjugant and if it is relevant in the field situations. Furthermore, additional data on the half-lives of the Cry proteins are required in order to exclude the chronic toxicity to bees. Information on infectivity and pathogenicity to honey bees and bumble bees must be submitted. The RMS is of opinion that if no further in-

				formation is provided a restriction sentence is necessary prohibiting the application of the product when the crop is flowering.
	It is considered that, on the basis of the assessment of Community or internationally agreed test guidelines, the substance **HAS** endocrine disrupting properties that may cause adverse effects on non-target organisms.		x	
	Linked to the consideration of the endocrine properties immediately above. It is considered that the exposure of non-target organisms to the active substance in a plant protection product under realistic proposed conditions of use is negligible.	x		
	It is considered that it is established following an appropriate risk assessment on the basis of Community or internationally agreed test guidelines, that the use under the proposed conditions of use of plant protection products containing this active substance, safener or synergist: — will result in a negligible exposure of honeybees, or — has no unacceptable acute or chronic effects on colony survival and development, taking into account effects on honeybee larvae and honeybee behaviour.		x	The additional data is required in order to assess if the potential risk to bumblebees seen in the study by Mommaerts et al. (2009) is relevant for the current transconjugant and if it is relevant in the field situations. Furthermore, additional data on the half-lives of the Cry proteins are required in order to exclude the chronic toxicity to bees. Information on infectivity and pathogenicity to honey bees and bumble bees must be submitted. The RMS is of opinion that if no further information is provided a restriction sentence is necessary prohibiting the application of the product when the crop is flowering.
Residue definition				
		Yes	No	
	It is considered that, where relevant, a residue definition can be established for the purposes of risk assessment and for enforcement purposes.			No residue definition is required.
Fate and behaviour concerning groundwater				
		Yes	No	
	It is considered that it has been established for one or more representative uses, that consequently after application of the plant protection product consistent with realistic conditions on use, the predicted concentration of the active substance or of metabolites, degradation or reaction products in groundwater complies with the respective criteria of the uniform principles for evaluation	X		Bt and spores are not considered mobile in soil. Leaching is not expected.

	and authorisation of plant protection products referred to in Article 29(6) of Regulation 1107/2009.			

3.1.2 Proposal – Candidate for substitution

Candidate for substitution				
		Yes	No	
	It is considered that the active substance shall be approved as a candidate for substitution		X	Not persistent for fate. No criteria are met for human toxicology

3.1.3 Proposal – Low risk active substance

Low-risk active substances				
		Yes	No	
	It is considered that the active substance **shall be considered of low risk.** An active substance which is a micro-organism may be considered as being of low-risk unless at strain level it has demonstrated multiple resistance to anti-microbials used in human or veterinary medicine. Baculoviruses shall be considered as being of low-risk unless at strain level they have demonstrated adverse effects on non-target insects.'	X		To be in compliance with the EFSA guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance (*EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP); Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. EFSA Journal 2012;10(6):2740. [10 pp.] doi:10.2903/j.efsa.2012.2740. Available online: www.efsa.europa.eu/efsajournal)* additional antibiotics were tested following standard procedures for antibiotic testing by deep agar diffusion tests using BD BBL Sensi-Disc Antimicrobial Susceptibility Test Discs from VWR (Cheng & Chen, 2016). Bta GC-91 is sensitive or at least intermediate susceptible to all antibiotics recorded in the EFSA guidance document for Bacillus spp. used in feed additives (chloramphenicol, tetracycline, streptomycin, clindamycin, erythromy-cin, streptomycin, kanamycin, gentamycin and vancomycin)

3.1.4 List of studies to be generated, still ongoing or available but not peer reviewed

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going	Study on-going and anticipated date of completion	Study available but not peer-reviewed
3.1.4.1 GENERAL for all chapters				
Applicant: please check the reference list at each chapter. A lot of old articles already used in the previous DAR are mentioned with no references described in the text. RMS added some references in the text, please check. And please add other relevant references in the text. In case they are not used in the text please delete them in the reference list.				
3.1.4.2 Identity of the active substance or formulation				
At B.2.2.2 MA: No references have been provided concerning the mean biopotency and expression of the different Cry proteins of strain GC-91.	Relevant information	Not relevant	Not relevant	Not relevant
At B.2.6 MA: 1) A reference concerning the formally recognised members of the *Bacillus cereus* group should be provided. 2) No reference has been provided concerning the information that the actual contribution of *B. cereus* and *B. thuringiensis* to gastrointestinal and non-gastrointestinal diseases is currently unknown. 3) *B. mycoides*, *B. pseudomycoides* and *B. toyonensis* have not yet been described to	Relevant information	Not relevant	Not relevant	Not relevant

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going	Study on-going and anticipated date of completion	Study available but not peer-reviewed
have the potential to cause foodborne diseases. Confirmation is needed.				
At B.2.7 MA: Concerning the literature search in regard to whether or not Bta GC-91 may transfer genetic material to other micro-organisms after field application the search strategy included transformation, transduction, and conjugation but not transposon. Besides transformation (uptake of exogenous DNA), conjugation (exchange of DNA between bacterial cells) and transduction (mediated by viruses) another driver for DNA-exchange between two bacterial cells are so-called integrative and conjugate elements (ICEs). These mobile genetic elements which are also referred to as conjugative transposons are able to switch their location within the genome but can also be transferred into a recipient genome via conjugation. ICEs often contain antibiotic-resistance, symbiosis or virulence genes, thereby contributing to bacterial evolution by conferring new phenotypes to their recipients. This point should be addressed.	Relevant information	Not relevant	Not relevant	Not relevant
Ad literature search B.2 MA: 1) References relied on, 4. Search methods and results, p. 85-87: There are some incon-	Information necessary	Not relevant	Not relevant	Not relevant

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going	Study on-going and anticipated date of completion	Study available but not peer-reviewed
sistencies regarding the literature search. In general there are several synthax errors in regard to the brackets used in the search strategy. This may indicate that the literature search was not adequately performed but may perhaps also not have influenced the search results. While the search strategy concerning genetic stability contains in total six opening brackets and seven closing ones on page 86 it contains 5 opening brackets and 6 six closing ones on page 87 (Table 4-1). Similarly, the search strategy for the development of resistances contains in total only two opening brackets but three closing ones. 2) References relied on, 4. Search methods and results, Table 4-1, p. 87: Please indicate what FT stands for! Full title? Full text? Any other? 3) References relied on, 4. Search methods and results, Table 4-1, p. 87: Concerning Search 4, shouldn't it be 1 AND 2 NOT 3 (instead of 2 NOT 3)? According to the search method outlined before the search strategy was Bacillus thuringiensis AND (aizawai OR kurstaki) AND […]. If the search strategy here would have been 2 NOT 3 as indicated all the keywords to be excluded from the search would still have been part				

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going	Study on-going and anticipated date of completion	Study available but not peer-reviewed
of the search results under Search 1. 4) References relied on, 4. Search methods and results, Table 4-2, p. 88: Search strategy 4 here clearly differs from Table 4-1 (1 NOT 2 instead of 2 NOT 3). Again, shouldn't it be 1 AND 2 NOT 3? If the search strategy here would have been 1 NOT 2 as indicated all the results from search 2 (concerning the subspecies kurstaki) would have been excluded. 5) References relied on, 4. Search methods and results, Table 4-2, p. 88: Something is strange about the total number of results. If search strategy 4 is indeed 1 AND 2 NOT 3 then the total number of results amounts to 64 + 17 + 241 = 322 already after removing of duplicates. How then can the total number be even higher than that (i.e. 330) if in addition duplicate references due to searches for the two different subspecies of Bt were deleted from the list? As the search strategy as outlined is somewhat confusing clarification is needed and these 5 points should be addressed.				
3.1.4.3 Physical and chemical properties of the active substance and physical, chemical and technical properties of the formulation				

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going	Study on-going and anticipated date of completion	Study available but not peer-reviewed
3.1.4.4 Data on uses and efficacy				
3.1.4.5 Data on handling, storage, transport, packaging and labelling				
3.1.4.6 Methods of analysis				
3.1.4.7 Toxicology and metabolism				
Ad B.6.2.2: The reference of the in vivo study in somatic cells should be indicated and more information in the summary should be presented.	Information relevant	Not relevant	Not relevant	Not relevant
3.1.4.8 Residue data				
3.1.4.9 Environmental fate and behaviour				
3.1.4.10 Ecotoxicology				
Please address the possible risk to honey bees bumble bees from the current applications (see the above sections).				

3.1.5 Issues that could not be finalised

An issue is listed as an issue that could not be finalised where there is not enough information available to perform an assessment, even at the lowest tier level, for the representative uses in line with the Uniform Principles, as laid out in Commission Regulation (EU) No 546/2011, and where the issue is of such importance that it could, when finalised, become a concern (which would also be listed as a critical area of concern if it is of relevance to all representative uses).

Area of the risk assessment that could not be finalised on the basis of the available data	**Relevance in relation to representative use(s)**
Risk to honey bees and bumblebees	*All uses*

3.1.6 Critical areas of concern

An issue is listed as a critical area of concern:
(a) where the substance does not satisfy the criteria set out in points 3.6.3, 3.6.4, 3.6.5 or 3.8.2 of Annex II of Regulation (EC) No 1107/2009 and the applicant has not provided detailed evidence that the active substance is necessary to control a serious danger to plant health which cannot be contained by other available means including non-chemical methods, taking into account risk mitigation measures to ensure that exposure of humans and the environment is minimised, or
(b) where there is enough information available to perform an assessment for the representative uses in line with the Uniform Principles, as laid out in Commission Regulation (EU) 546/2011, and where this assessment does not permit to conclude that for at least one of the representative uses it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater or any unacceptable influence on the environment.

An issue is also listed as a critical area of concern where the assessment at a higher tier level could not be finalised due to a lack of information, and where the assessment performed at the lower tier level does not permit to conclude that for at least one of the representative uses it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater or any unacceptable influence on the environment.

Critical area of concern identified	**Relevance in relation to representative use(s)**
None.	

3.1.7 Overview table of the concerns identified for each representative use considered

(If a particular condition proposed to be taken into account to manage an identified risk, as listed in 3.3.1, has been evaluated as being effective, then 'risk identified' is not indicated in this table.)

Representative use		Use "A" (apple)	Use "B" (grape)	Use "C" (tomato)	Use "D" (turf,sports)
Operator risk	Risk identified				
	Assessment not finalised				
Worker risk	Risk identified				
	Assessment not finalised				
Bystander risk	Risk identified				
	Assessment not finalised				
Consumer risk	Risk identified				
	Assessment not finalised				
Risk to wild non target terrestrial vertebrates	Risk identified				
	Assessment not finalised				
Risk to wild non target terrestrial organisms other than vertebrates	Risk identified				
	Assessment not finalised	x	x	x	x
Risk to aquatic organisms	Risk identified				
	Assessment not finalised				
Groundwater exposure active substance	Legal parametric value breached				
	Assessment not finalised				
Groundwater exposure metab-olites	Legal parametric value breached				
	Parametric value of 10 µg/L(a) breached				
	Assessment not finalised				
Comments/Remarks					

The superscript numbers in this table relate to the numbered points indicated within chapter 3.1.5 and 3.1.6. Where there is no superscript number, see level 2 for more explanation.

(a): Value for non relevant metabolites prescribed in SANCO/221/2000-rev 10-final, European Commission, 2003

3.1.8 Area(s) where expert consultation is considered necessary

It is recommended to organise a consultation of experts on the following parts of the assessment report:

Area(s) where expert consultation is considered necessary	**Justification**
None.	

3.1.9 Critical issues on which the Co RMS did not agree with the assessment by the RMS

Points on which the co-rapporteur Member State did not agree with the assessment by the rapporteur Member State. Only the points relevant for the decision making process should be listed.

Issue on which Co-RMS disagrees with RMS	**Opinion of Co-RMS**	**Opinion of RMS**
Whether commercial Bt strain such as Bta GC-91 can be compared to a pathogenic *B. cereus* strain and that a prediction of a safety level for commercial Bt strains based on information of pathogenic *B. cereus* isolates is reasonable. RMS would like to ask the other member states to look carefully to this issue.	The assumptions presented by the RMS are not sufficient to demonstrate that *B. cereus* strains differ significantly from commercial Bt strains. Since no new data was presented in the RAR, the available data is considered as not sufficient to distinguish between both species in terms of their potential to induce foodborne diseases. Therefore the conclusions drawn by EFSA´s BIOHAZ panel are still considered valid and have to be considered in the course of risk assessment, because based on EFSA´s BIOHAZ Panel opinion on the presence of Bacillus cereus and other Bacillus spp. in foods (EFSA Journal 2016;14(7):4524) food-borne diseases were associated with concentrations above 10^5 CFU/g, which was also considered relevant for B. thuringiensis. As a consequence a comprehensive risk assessment considering the CFU threshold level and the CFU levels resulting from the representative uses is still outstanding. Since no experimental data is available on the CFU after application of Bt, on the potential amount of toxins expressed under natural conditions, and on the decline under field conditions, there is a requirement for residue trials. They should address the residues at harvest but also at the point of consumption, as it is possible that the levels of CFU increase during transport, processing etc. Available analytical methods for monitoring are not	RMS is of the opinion that commercial Bt strain such as Bta GC-91 cannot be compared to a pathogenic *B. cereus* strain. The traits responsible for a potential health risk to consumers are highly strain specific. Available strain-specific studies of Bta GC-91 in test animals confirm the absence of toxicity and pathogenicity. Therefore, a prediction of a safety level for strain GC-91 based on information of pathogenic *B. cereus* isolates is not reasonable. The EFSA BIOHAZ panel indicates that most cases of foodborne outbreaks caused by the *B. cereus* group have been associated with concentrations above 10^5 CFU/g and that the levels of *B. cereus* that can be considered as a risk for consumers might be also valid for *B. thuringiensis*. However, as already stated above, this approach is not justified as pathogenic *B. cereus* strains differ significantly from commercial Bt strains in the physiological requirements, environmental behaviour and their toxigenic and pathogenic potential. Based on available information it can be concluded that the risk for consumers due to possible exposure of Bta CG-91 is acceptable. This is confirmed by a lack of case reports in which commercially-used *B. thuringiensis* is directly associated with food poisoning (Bta has been used in agriculture for approximately one century; Bta GC-91 has been approved e.g. in The Netherlands for more than twenty

Issue on which Co-RMS disagrees with RMS	Opinion of Co-RMS	Opinion of RMS
	suitable to unequivocally distinguish between closely related strains within the group of Bacillus spp. With reference to B.5, strain specific analytical methods need be developed under the data requirements defined in Reg. (EU) 1107/2009, if commercial Bt strains such as Bta GC-91 are assessed in a different way as other Bacillus spp. in foods.	years).

3.2 Proposed decision

[redacted]

3.3 Rational for the conditions and restrictions to be associated with the approval or authorisation(s), as appropriate

3.3.1 Particular conditions proposed to be taken into account to manage the risk identified

Proposed condition/risk mitigation measure	Relevance in relation to representative use(s)
[redacted]	[redacted]

3.4 Appendices

3.4.1 Guidance documents used in this assessment

Guidances applicable at the time of submission of the additional dossier were used in this assessment.

3.5 Reference list

List [in the conventional format] any references specifically cited in Volume 1 (i.e references to underpinning documents such as PPR-Panel Opinions, EFSA conclusions, national documents etc.).