Renewal
Assessment Report

Bacillus thuringiensis subsp. aizawai strain GC-91

- Agree 50 WG -

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Volume 3 – B.8 Fate and behaviour in the environment

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Rapporteur Member State: The Netherlands

Co-Rapporteur Member State: Germany

Version history

When	What
July 2018	Initial RAR

Table of contents

B Summary, evaluation and assessment of the data and information

B.8 Fate and behaviour in the environment

Bacillus thuringiensis subsp. *aizawai* GC-91 (in the following abbreviated as Bta GC-91) was one of the existing active substances covered by the Regulation (EC) No 2229/2004 on the implementation of the fourth stage of the program of work referred to in Article 8(2) of Council Directive 91/414/EEC. In Annex I to Regulation (EC) No 2229/2004 the Commission designated Italy as rapporteur Member State to carry out the assessment of Bta GC-91 on the basis of a dossier submitted by the notifier Mitsui AgriScience International SA/NV. In accordance with the provisions of Article 22(1) of Regulation (EC) No 2229/2004, Italy submitted in November 2007 to the EFSA the draft assessment report, including, as required, a recommendation concerning the possible inclusion of Bta GC-91 in Annex I to the Directive. The Commission examined the draft assessment report, the recommendations by the rapporteur Member State and the comments received from other Member States in consultation with experts from a certain number of Member States. The Commission referred on 11 July 2008 a draft review report to the Standing Committee on the Food Chain and Animal Health, for final examination. The draft review report was finalised in the meeting of the Standing Committee on 11 July 2008. Subsequently Regulation (EC) No 1107/2009 repealed and replaced Directive 91/414/EEC and the active substance Bta GC-91, was deemed to be approved under that Regulation and included in the Annex to Regulation (EC) No 540/2011. EFSA delivered its conclusions on *Bacillus thuringiensis* ssp. *aizawai* (strains ABTS-1857, GC-91) on the 19 December 2012 (published January 2013). Based on this new information available, no need to change the conditions of approval of Bta GC-91 was identified. The Commission filed on 13 December 2013 an updated review report for Bta GC-91 to the Standing Committee on the Food Chain and Animal Health for examination.

The approval of Bta GC-91 under the Regulation (EC) No 1107/2009 expires 30 April 2019. In accordance with the same Regulation the original notifier Mitsui AgriScience International SA/NV has filed to the Commission an application for the renewal of the approval of the active substance Bta GC-91 on 30 April 2016. In accordance with Regulation (EU) 2016/183 the notifier is submitting to the designated RMS The Netherlands, the co-RMS Germany as well as to EFSA and the Commission a dossier for renewal of Bta GC-91 considering the deadline stated in SANTE-2016-10616–rev. 3.

Bta GC-91 is a transconjugant strain between 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 first assessment of the strain proved that it does not have any harmful effects on human or animal health or on groundwater or any unacceptable influence on the environment. The overall conclusion from EFSA (2013) confirms that no critical areas of concern are identified within the framework of the use which was supported.

The representative formulation for original approval of Bta GC-91 was Agree 50 WP. For renewal under Regulation (EC) 1107/2009 a WG formulation, Agree 50 WG, was chosen containing the same amount of Bta GC-91 as Agree WP (50%) and very similar co-formulants. Both products have the same biopotency of 25000 IU/mg. The maximum CFU content in Agree 50 WG is 3.3×10^{13} CFU/kg product. Due to the similarity of the products it is justified to use data for Agree 50 WP also for the evaluation of Agree 50 WG. In addition, the manufacturing process of Bta GC-91 has not been changed since original approval and all data previously submitted for the strain are considered fully applicable for the current evaluation.

As agreed with the RMS, besides new information, the submitted dossier includes all data, which have been presented in the DAR (May 2007) and DAR addendum (February 2013). This information is marked grey.

Representative uses chosen for renewal of Bta GC-91 cover control of *Lobesia botrana* and *Eupoecillia ambiguella* in grapes (as for original approval) as well as *Cydia pomonella* in pome fruits and *Spodoptera* spp. in turf as field uses, as well as *Tuta absoluta* in tomato in the greenhouse. Both, use by professionals and non-professionals is intended. The maximum intended application rate is 2 kg with 6 subsequent applications at an interval of 7 days.

It is considered that the Critical GAP of Agree 50 WG chosen for the renewal of the active substance Bta GC-91 covers worst case exposure scenarios for human, non-target organisms and the environment.

Critical GAP of Agree 50 WG for renewal of Bta GC-91

Crop	**F G or I**	**Pest**	**Application**			**Application rate**		
			Method / Kind	**Growth stage of crop**	**Max. number (min. interval between applications)** **a) per use** **b) per crop/ season**	**Kg product / ha** **a) max. rate per appl.** **b) max. total rate per crop/season**	**g as/ha IU/ha** **a) max. rate per appl.** **b) max. total rate per crop/season**	**Water L/ha** **min / max**
Pome fruits	F	*Cydia pomonella*	Foliar spray	BBCH 53-99	a) 6 (7) b) 6 (7)	a) 2.0 b) 12.0	a) 1000 / 5×10^{10} b) 6000 / 3×10^{11}	1000-1500
Grapes	F	*Lobesia botrana,* *Eupoecilia ambiguella*	Foliar spray	BBCH 53-99	a) 6 (7) b) 6 (7)	a) 2.0 b) 12.0	a) 1000 / 5×10^{10} b) 6000 / 3×10^{11}	200-1200
Tomato	G	*Tuta absoluta*	Foliar spray	BBCH 12-89	a) 6 (7) b) 6 (7)	a) 2.0 b) 12.0	a) 1000 / 5×10^{10} b) 6000 / 3×10^{11}	500-1500
Turf, Sports	F	*Spodoptera* spp.	Foliar spray	BBCH 12-89	a) 6 (7) b) 6 (7)	a) 2.0 b) 12.0	a) 1000 / 5×10^{10} b) 6000 / 3×10^{11}	1000-1500

Biopotency of Agree 50 WG: 25000 IU/mg
Max. CFU content in Agree 50 WG: 3.3×10^{13} CFU/kg

B.8.1 Persistence and multiplication

B.8.1.1 Soil

Fate and behaviour in soil

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

Based on information derived from studies and the published literature on the species *Bacillus thuringiensis* and the strain *B. thuringiensis* subsp. *aizawai* GC-91, the environmental fate and population dynamics of this bacterium can be summarized as follows:

Bacillus thuringiensis is a ubiquitous bacterium occurring worldwide, mainly in soils as well as on insects and on plant surfaces. *B. thuringiensis* belongs to the spore forming bacteria of the family Bacilliacea. Dormant spores of *B. thuringiensis* can persist for long in the environment, but are metabolically inactive. Its application in the soil will only temporally and locally alter the natural population of the species, which will slowly return to its so called dynamic equilibrium (soil homeostasis). This is confirmed by a study by Konecka et al (2014, 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.

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 and < 21days for Cry3Bb1.

Predicted environmental population density in soil

In order to perform a risk assessment for non-target organisms the actual population of *B. thuringiensis*. subsp. *aizawai* GC-91 spores is calculated for soil, based on the maximum accumulated application rate of 12 kg product/ha in pome fruits, grapes and turf upon foliar application, assuming 6 treatments of 2 kg/ha and as a worst case no degradation between the multiple applications. The resultant amount of active substance will be related to the top 5 cm of soil to achieve the highest theoretical soil population.

For the calculation the content of 500 g a.s./kg product has been considered. In addition, the PED value is indicated in CFU/kg soil dry weight (dw), based on a maximal content of 3.3×10^{13} CFU/kg.

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.

The resultant actual density of viable spores of *B. thuringiensis* considering the worst-case scenario is presented in **Table B.8.1.1-01**.

Table B.8.1.1-01 Calculation of the predicted environmental density of Agree 50 WG and *B. thuringiensis* in soil (PED_{soil}) after 6 applications at 2 kg Agree 50 WG/ha

Accumulated application rate [kg product/ha]	**Rate [mg product/m²]**	**Soil depth [cm]**	**Bulk density [g/cm^3]**	**Initial PED related to soil depth [mg product/kg soil (dw)]**
12	1200	5.00	1.5	16.0
Accumulated application rate [kg a.s./ha]	**Rate [mg a.s./m²]**	**Soil depth [cm]**	**Bulk density [g/cm^3]**	**Initial PED related to soil depth [mg a.s./ kg soil (dw)]**
6.0	600	5.00	1.5	8.0
Accumulated application rate [CFU/ha]	**Rate [CFU/m²]**	**Soil depth [cm]**	**Bulk density [g/cm^3]**	**Initial PED related to soil depth [CFU/ kg soil (dw)]**
3.96×10^{14}	3.96×10^{10}	5.00	1.5	5.28×10^8

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.

B.8.1.2 Water

Fate and behaviour in water

Surface water

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

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

Predicted environmental density in natural waters

The envisaged field of use as a foliar treatment 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 (pome fruits and grapes) and downward (turf) application, each.

Table B.8.1.2-01 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)**	**Initial PED_{SW}**
Agree 50 WG	12 kg/ha	9.21	110.52 mg/m²	300 L/m²	368.4 µg/L
***Bacillus thuringiensis* subsp. *aizawai* GC-91**	6 kg/ha	9.21	55.26 mg/m²	300 L/m²	184.2 µg/L
	3.96×10^{14} CFU/ha		3.65×10^{9} CFU/m²		**1.22×10^{7} CFU/L**

a) Accumulated application rate, assuming no degradation between applications
b) Drift value for 6 applications in fruit crops (late)

Table B.8.1.2-02 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 grapes

	Application rate[a)]	**Relevant drift rate [%]**[b)]	**Amount reaching the water**	**Water volume (30 cm water layer)**	**Initial PED_{SW}**
Agree 50 WG	12 kg/ha	6.41	76.92 mg/m²	300 L/m²	256.4 µg/L
***Bacillus thuringiensis* subsp. *aizawai* GC-91**	6 kg/ha	6.41	38.46 mg/m²	300 L/m²	128.2 µg/L
	3.96×10^{14} CFU/ha		2.54×10^{9} CFU/m²		8.46×10^{6} CFU/L

a) Accumulated application rate, assuming no degradation between applications
b) Drift value for 6 applications in grapes (late)

Table B.8.1.2-03 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 turf

	Application rate[a)]	**Relevant drift rate [%]**[b)]	**Amount reaching the water**	**Water volume (30 cm water layer)**	**Initial PED_{SW}**
Agree 50 WG	12 kg/ha	1.64	19.68 mg/m²	300 L/m²	65.6 µg/L
***Bacillus thuringiensis* subsp. *aizawai* GC-91**	6 kg/ha	1.64	9.84 mg/m²	300 L/m²	32.8 µg/L
	3.96×10^{14} CFU/ha		6.49×10^{8} CFU/m²		2.16×10^{6} CFU/L

a) Accumulated application rate, assuming no degradation between applications
b) Drift value for 6 applications in field crops

The maximum PED_{SW} of **1.22×10^7 CFU/L** (corresponding to 368.4 µg Agree 50 WG/L and 148.2 µg a.s./L) is used for the risk assessments resulting from the application in orchards (pome fruits) with 6 × 2.0 kg Agree 50 WG/ha.

The RMS agrees with the PEDsw calculation as performed above by the notifier. The drift percentage used for the diferent crops and stages are based on the Rautmann drift values. The RMS has the opinion to use a TOX-SWA standard ditch of 210 l/m^2. This results in a 1.429 (300/210) higher PEDsw of **1.74×10^7 CFU/L** for the application in orchards.

Drinking water

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

B.8.1.3 Air

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 seeB.8.1.3) showed that re-aerosolisation may occur under a controlled indoor environment that simulated outdoor wind conditions. However, the fate in air for these spores will follow the same decline pattern.

B.8.2 Mobility

Ground water

From the information from the original evaluation of *B. thuringiensis* 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.

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

B.8.3 References relied on

No references submitted for this part.