Renewal Assessment Report

Bacillus thuringiensis subsp. *aizawai strain GC-91*

- Agree 50 WG -

Volume 3 – B.7 Residue data

July 2018

Rapporteur Member State: The Netherlands

Co-Rapporteur Member State: Germany

Version history

When	What
July 2018	Initial RAR

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Introduction

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.

As the manufacturing process of Bta GC-91 has not been changed since original approval, all data submitted for the original approval of the strain are considered fully applicable for the current evaluation.

Besides new information, the submitted dossier includes all data, which have been presented in the DAR (Jan 2008) and DAR addendum (Nov 2012). This information is marked grey with a clear indication where the information is originating from.

B.7 Residue data

No new information has been submitted with regard to residues of Agree. Previously submitted information on residues of formulations of *Bacillus thuringiensis* spores and crystals is still valid. Furthermore, the assessment is based on the information provided for the active substance.

For the purpose of the risk assessment for this section the worst-case exposure scenario is a foliar application in grapes with up to 6 applications at a dose rate of maximum 2 kg product/ha (1000 g *B. thuringiensis*/ha or 3.3×10^{13} CFU/ha), employed as critical use (**Critical GAP provided in introduction**).

Bacillus thuringiensis subsp. *aizawai* strain GC-91 is a transconjugant, whose parental strains (HD-191-A2 and HD-135-4S) were derived from the wild types HD-191 and HD-135. The strain is not genetically modified.

B. thuringiensis is a common, naturally occurring bacterium that is frequently isolated from soil, and from leaves, where it is regarded as a common part of the leaf microflora.

No toxicity or pathogenicity was observed in acute toxicity tests in mammals. *Bacillus thuringiensis* subsp. *aizawai* CG-91 induced no signs of toxicity at a dose of 9.4×10^{8} - 1.1×10^{10} CFU per kg bw.

B. thuringiensis subsp. *aizawai* is highly specific for larvae of Lepidoptera and does not affect other animals, including domestic animals and man, or plants.

Persistence of Bt on leaves is low. Half life of viable spores is about 1 day. Applied as a spray, the δ-endotoxins are rapidly degraded and endospores are rapidly inactivated when exposed to UV radiation (see other sections in Volume 3).

Following application of Agree according to GAP (**Table 8-1**), no accumulation of Bt on leaves will occur since it was shown that Bt spores are not able to germinate on leaves and fruit surfaces.

A worst case estimation of residues of *B. thuringiensis* subsp. *aizawai* CG-91 at harvest in CFU/g commodity was presented in the original DAR.

Based on the critical GAP of 0.8 kg/ha in grapes applied 4 times with an interval of 7 days, and a content in the product of 500 g/kg or 3 x 10^{13} CFU/kg or 25,000 IU/mg the following calculation can be done:

The parameters used to calculate the worst-case scenario for residual numbers of spores on grapevine leaves are

Concentration of Bt in the product (CFU/kg)	3×10^{13}
Application rate of formulated product (kg/ha)	1.6
Max. number of applications	4
Quantity of MPCA per year (kg/ha)	6.4
Max. cumulative population (CFU/ha)	1.92×10^{14}

The cumulative population distributed for ha is 1.92×10^{14} CFU/ha, when assuming no decrease in the population between applications.

Considering the "F*ocus groundwater scenarios in the EU review of active substances", Report of the FOCUS Groundwater Scenarios Workgroup, EC Document reference SANCO/321/2000 rev.2, 202pp, version 1.1, April 2002*," an interception factor of 0.85 for fully developed grapes can be used. So 85 % of the sprayed application is intercepted by grapevine leaves and grapes and 15 % reaches the soil surface.

Consequently, very likely 1.6×10^{14} CFU are applied on grapevine plants per ha.

Max. cumulative population [CFU/ha]	1.92×10^{14}
Interception factor	0.85
Population applied on vine plants [CFU/ha]	1.6×10^{14}

The average yield for grape is assumed to be 11000 kg/ha in Germany (northern Europe) and might be considered 5500 kg (medium) in the southern European countries.

Population applied on vine plants (CFU/ha)	1.6×10^{14}
Yield (kg/ha) by Germany production	11000
Yield (kg/ha) by southern Europe (Italy)	5500
Max. estimated concentration on product (CFU/g)	1.48×10^{7}
Max. estimated concentration on product (CFU/g)	2.96×10^{7}

This results in a final content of 1.48×10^{7} CFU/g grape in the most favourable case (northern Europe) or twice as much in the worst case (southern Europe). However, it is also correct to consider that this calculation does not reflect the realistic conditions where the stability of Bt spores on leaves and berries is very limited.

When Bt is applied as a spray, the endospores are rapidly inactivated and the endotoxins are rapidly degraded when exposed to UV radiation; the half life of viable spores is 1- 2 days. Also considering that Bt spores are not able to germinate on leaves and fruits surface, no accumulation of Bt on leaves will occur.

The last application results in a spore concentration of 3.7×10^{6} CFU/g or $7,4 \times 10^{6}$ CFU/g immediately after application. The parameters used to calculate the degradation of Bt spore numbers on grapevine leaves are:

Concentration of Bt in the product [CFU/kg]	3×10^{13}
Application rate of product (kg/ha)	1.6
Number of applications	1
Population after last application (CFU/ha)	4.8×10^{13}
Interception factor	0.85
Population applied on vine plants (CFU/ha)	4.08×10^{13}
Yield (kg/ha) in Germany	11000
Yield (kg/ha) in Italy	5500
Population on product (CFU/g) in Germany	3.7×10^{6}
Population on product (CFU/g) in Italy	$7,4 \times 10^{6}$

Assuming a half-life of 24 hours for Bt, it is possible to consider a period of time of 14 days (336 hours), corresponding to 14 "half-life periods" for a *B. thuringiensis* population, a "safety period" to reduce the population of *B. thuringiensis* to a concentration of the order 10^3 CFU/g;

An update based on current GAP is reported in **Table 8-1**. For this calculation it is considered that for an application in grapes, all applied product is deposited on the plant as it is not justified to use interception values derived for chemicals and derived to estimate the amount of product reaching the soil, for the calculation of residue on crop. Furthermore, in the calculation it is assumed that all of the *B. thuringiensis* subsp. *aizawai* CG-91 that reaches the plant is deposited on the fruit and nothing is deposited on the leaves like in the DAR calculation.

In the initial calculation it is also assumed that there is no decrease in population between applications.

Table 8-1 Estimation of residues of *B. thuringiensis* subsp. *aizawai* CG-91

Concentration of Bt in the product (CFU/kg)	3.3×10^{13} CFU/kg
Application rate (kg/ha)	2 kg/ha
Application rate (CFU/ha)	6.6×10^{13} CFU/ha
Number of applications	6
Max cumulative population (CFU/ha)	3.96×10^{14} CFU/ha
Interception	Not considered
Population applied on grapes (CFU/ha)	3.96×10^{14} CFU/ha
Yield grapes in Germany*	11000 kg/ha $= 1.1 \times 10^7$ g/ha
Max. estimated concentration on harvested product	3.6×10^7 CFU/g

** The link to FAO gives data on tonnes vine grapes per country that show the France has the highest number, however, in the calculation the value is in tonnes/ha. The amount of hectares in both France and Italy is larger than in Germany, exact values however are not reported and therefore the calculation cannot be made and only the yield grapes in Germany are described.*

The values in **Table 8-1** are based on the worst-case assumptions and calculation based on cumulative application rate. In the original DAR (2011) a half-life time for *B. thuringiensis* subsp. *aizawai* CG-91 of less than 24 hours on leaves from maize and beans was derived. Therefore, in refined calculations a half-life time of 24 hours is supposed. The spray is applied with an interval of minimum 7 days.

After the first application the resulting amount of *B. thuringiensis* subsp. *aizawai* CG-91 is 6×10^6 CFU/g commodity (3.3×10^{13} CFU/kg × 2 kg/ha/1.1×10^7 g/ha). Using a half-life time of 24 hours the amount of *B. thuringiensis* subsp. *aizawai* CG-91 is 4.7×10^4 CFU/g after 7 days (see volume 3 B.2.5 MA describing that at leaves, half-life times are notably shorter ranging between 16 and 38 hours with more or less complete disappearance recorded between 15 and 60 days after application). Using the Multiple Application Method (EFSA Journal 2014;12(10):3874) to calculate, after application number 6, the total amount of Bta CG-91 is 6×10^6 CFU/g commodity directly after the 6^{th} application (based on an MAF of 1) and 4.7×10^4 CFU/g commodity 7 days after the last application.

Based on the backgrounds levels of max 6 x 10^4 CFU/g (presented in Table 2.1.2-1 in volume 3 B.2.1.2) levels of *B. thuringiensis* subsp. *aizawai* CG-91 decline following application to background levels of *B. thuringiensis* subsp. *aizawai* CG-91 by day 7. Therefore, no PHI is established.

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.

In conclusion, based on the information presented, further information on viable and non-viable residues of Bta CG-91 in treated food/feed stuffs is not considered to be necessary.

As the active substance is microbial in nature and in light of the information presented it is requested that *Bta CG-91* is included in Annex IV of Regulation 396/2005 as there is no residue of concern from the use of the active substance and it therefore should be exempted from establishing an MRL.

Reference list

No new references are submitted