Renewal
Assessment Report

Bacillus thuringiensis ssp. aizawai strain ABTS-1857

- XenTari® WG -

Volume 3 – B.5 Analytical methods

Rapporteur Member State: The Netherlands

Co-Rapporteur Member State: Germany

Version history

When	What
September 2018	Initial RAR

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B.5 Analytical methods

B.5.1 Methods for the analysis of the preparation

B.5.1.1 Methods for the identification and the determination of the content of the micro-organism(s) in the preparation

The determination of the content of the micro-organism in the preparation is determined by viable cell counts; measured in terms of its biopotency.

A bioassay based on the quantal dose response of four-day post-hatch *Trichoplusia ni* larvae to the test substance incorporated into an agar-based diet is described below. The analysis of lepidopteran biopotency was originally referred to in the DAR (2007), IIM 1.4.3/03, Martinat (1990). Further validation has been provided below.

Report:	KMP 5.1.1/01, Wicker, N. (2016)
Title:	Method validation of bioassay potency tests for the Cabbage Looper, *Trichoplusia ni*
Document No.	Laboratory Project ID: S16-01397 Eurofins Agroscience Services, Inc., Mebane, NC 27302
Guidelines:	US EPA OPPTS 885.1400 and 885.1500
GLP:	Yes

Principle of Method:
Four treatments: one reference standard (RS), Btk technical powder, one [redacted] check sample and two test substances [redacted] (TS1) and Xen Tari WG (TS2) were tested at six concentrations with an untreated control (UTC) for each concentration and for each replicate. Only the results of TS2 are reported below.
The seven final test concentrations in deionised water for all replicates of TS2 were 37500, 58125, 21000, 15750, 12000, 90000 and 0 µg/mL. The seven final test concentrations for the RS were 12718.750, 9539.063, 7122.500, 5341.875, 4070.000, 3052.200 and 0.0 µg/mL. The seven final test concentrations for the CS were 12656.250, 9492.188, 7087.500, 5315.625, 4050.00, 3037.50 and 0.00 µg/mL.
Specified volumes of each test concentrations were incorporated into bottles of molten diet (90 mL) with the appropriate amount of deionised water (0 to 10.0 mL). The bottles were then shaken and the contents poured onto three separate petri dishes. Once cooled and solidified, 10 larvae were placed onto each petri dish and covered with a lid. The petri dishes were then placed in an environmentally controlled chamber for 64-68 hours. At this timepoint, the insects were evaluated for mortality. Mortality was declared if the cabbage looper larvae did not respond to probing. The response (larval mortality) was analysed by weighted Probit analysis and an LC50 value calculated. The activity of TS2 was expressed as potency in International Units (IU) per mg of material relative to the RS.

Specificity:

An untreated control (UTC) was prepared for each concentration and each replicate. No interference from any formulants or impurities was noted.

Linearity:

A method (dose) response pattern was observed for all replicates over the range 21562.5 to 0.0 µg/mL for [redacted] (TS1). LC_{50} values calculated using the Probit analysis program PoloPlus were within

the range of doses tested.

Accuracy and Repeatability (Precision):

Mean potencies for XenTari WG ranged from 18800-22660 ITU/mg with an overall mean of 20698 ITU/mg. The accuracy of the method was determined by comparing the potency obtained in this study for XenTari WG against its target potency of 25,530 IU/mg. This was calculated to be 88%.

Mean potencies for the check sample, [redacted] ranged from [redacted] ITU/mg with an overall mean of [redacted] ITU/mg. The assigned potency for [redacted] is [redacted] IU/mg and therefore the % accuracy was determined to be [redacted].

Table 1: Calculated Potency of [redacted] (TS1) and [redacted] (CS)

Potency[1] (ITU/mg)		
Replicate	**TS2 XenTari WG**	[redacted]
1	19054	[redacted]
2	21869	[redacted]
3	18800	[redacted]
4	21835	[redacted]
5	22660	[redacted]
6	19967	[redacted]
Mean	**20698**	[redacted]
StDev	1634	[redacted]
%CV	**8%**	[redacted]
N	6	[redacted]
% Accuracy	**88%**	[redacted]

Conclusion:

The determination of the content of the micro-organism in the preparation is measured in terms of its biopotency.

A bioassay based on the quantal dose response of four-day post-hatch *Trichoplusia ni* larvae to the test substance incorporated into an agar-based diet has been validated as a method to determine the biopotency of the preparation.

The study meets the requirements of Part B of Regulation (EU) No 284/2013 and no further data are required for this endpoint.
The methods to show that contaminating micro-organisms are controlled to an acceptable level are summarized in B.5.1.7

B.5.1.2 Methods to establish regular control of the preparation to show that it does not contain other organisms than the indicated ones and to establish uniform

Relevant data on microbial contaminants have been provided for the technical material. See volume 3, B.5 MA.

B.5.1.3 Methods to identify any contaminating micro-organisms of the preparation

Relevant data on microbial contaminants have been provided for the technical material. See volume 3, B.5 MA.

B.5.1.4 Methods for the determination of relevant impurities or metabolites in the manufactured material

See volume 3, B.5 MA.

B.5.1.5 Methods used to determine the storage stability and shelf life of the preparation

See volume 3, B.5
MA.

B.5.2 Methods to determine and quantify residues (viable or non-viable)

For a new identification method at strain level it is referred to Vol 3 MA B.5.1.1/2. No residue analytical methods are required

Bacillus thuringiensis subsp. *Aizawai* ABTS-1857 such as all Bta 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. For more information, please refer to information provided for the MA in Section M-MA, Section 1, Point MA 2.8. In principle, strain specific methods are available for monitoring of the strain on treated plants. Please refer to Volume 4 and volume 3 B.5 MA..

B.5.3 References relied on

Reference list, by Data Point Number

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KMP 5.1.1/01	Wicker, N.	2016	Method validation of bioassay potency tests for the Cabbage Looper, Trichoplusia ni Laboratory Project ID: S16-01397 Eurofins Agroscience Services, Inc., Mebane, NC 27302	N			