Renewal
Assessment Report

Bacillus thuringiensis ssp. aizawai strain ABTS-1857
-XenTari WG-

Volume 3 – B.9 Effects on non-target organisms

Rapporteur Member State: The Netherlands

Co-Rapporteur Member State: Germany

Version history

When	What
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Introduction

Bacillus thuringiensis subsp. *aizawai* strains ABTS-1857, GC-91 were included in Annex I to Directive 91/414/EEC (2008/113/EC) on 1 May 2009 pursuant to Article 24b of the Regulation (EC) No 2229/2004 and has subsequently been deemed to be approved under Regulation (EC) No 1107/2009 in accordance with Commission Implementing Regulation (EU) No 540/2011 as amended by Commission Implementing Regulation (EU) No 541/2011.

European Food Safety Authority (EFSA) revised the draft review report submitted by the European Commission and EFSA's conclusion was published in the peer review (EFSA Journal 2013; 11(1): 3063).

Italy was designated rapporteur Member State and the DAR was issued in 2008. Parts of the original DAR are presented in this document.

The plant protection product XenTari® WG is considered representative of uses of the active substance *Bacillus thuringiensis* subsp. *aizawai* strain ABTS-1857 for the purposes of renewal of the approval of the active substance under EC Regulation 1107/2009 according to Regulation EU 283/2013.

Bacillus thuringiensis are rod-shaped, gram positive, aerobic bacteria, approximately 1 by 5 microns long. *Bacillus thuringiensis* is a naturally occurring, spore-forming bacteria, which is used as a microbial pest control agent (MPCA). Upon sporulation, *Bacillus thuringiensis* forms crystals of proteinaceous insecticidal δ-endotoxins (also referred to in the literature as crystalline proteins, cry proteins, Cry1Ab protein, Cry1Ab toxins, insecticidal crystal proteins (ICPs), parasporal crystals, parasporal protein-crystal, parasporal crystalline inclusions), which are encoded with *Cry* genes. These crystalline proteins consist predominantly of a single species of polypeptide called protoxins. Once ingested by a susceptible insect (*e.g.* Lepidoptera), the protoxins are solubilised under the alkaline conditions of the insect midgut; the protoxins are proteolytically activated by proteases to become activated Cry toxins. The activated Cry toxins then bind readily to specific receptors on the apical brush border of the midgut microvillae of susceptible insects, leading to cell disruption and consequently death of the insect.

There are several different subspecies (or serovars/serotypes) of *Bacillus thuringiensis*. The fate and behaviour of *Bacillus thuringiensis* subsp. *aizawai* strain ABTS-1857 is assessed. *Bacillus thuringiensis* subsp. *aizawai* is the MPCA component of the product XenTari® WG; a summary of the XenTari® WG GAP is presented in Table B.9-1 below.

Table B.9-1 Summary of critical GAP for XenTari WG

Crop and/or situation	Formulation conc. of MPCA	Application		Application rate per treatment			PHI[1] (days)
		Growth stage & season / Method	Number / Interval between applications (min)	MPCA g/hL min - max	Water L/ha max	MPCA kg/ha max	
Outdoor fruit vegetables (pepper)	54% w/w Approx. $5x10^{13}$ cfu/kg	BBCH 09-BBCH 92 (May-Oct) Start when larvae hatch (L1) Spray	1 – 8 applications (1 - 3 per generation of pest) 6 – 10 day minimum interval (typically 7 days)	0.027 – 0.054 kg a.s. /hL (0.050 – 0.100 kg f.p./hL)	Typically 500-1000 L/ha	0.270–0.540 kg a.s./ha. (0.500-1.0 kg f.p./ha) Approx. $5x10^{13}$ cfu/ha	0
Indoor fruit vegetables (pepper)	54% w/w Approx. $5x10^{13}$ cfu/kg	BBCH 09-BBCH 92 (Jan-Dec) Start when larvae hatch (L1)	1 – 7 applications, (Typically 1 - 3 per generation of pest) Typically 7 days minimum interval	0.027 – 0.054 kg a.s./hL (0.050-0.100 kg f.p./hL)	400–1000 Typically 1000 L/ha	0.270 – 0.540 kg a.s./ha. (0.500–1.0 kg f.p./ha) Approx. $5x10^{13}$ cfu/ha	0

[1] pre-harvest interval

B.9.1 Effects on birds

B.9.1.1 Toxicity to birds

No studies are available assessing the effect of the representative formulation, XenTari® WG, on birds. The ingredients of the preparation XenTari® WG are inert and are not expected to present any hazards to the environment. Therefore, studies and information on the microbial pest control agent, BTa ABTS-1857, are considered applicable and relevant with regard to the evaluation of effects on birds of the formulated product. The ecotoxicological effects of BTa ABTS-1857 on birds were evaluated during the Annex I inclusion. Experiments on toxicity and pathogenicity of the MPCA were performed on mallard duck (*Anas platyrhynchos*) and bobwhite quail (*Colinus virginianus*). Full details of these studies are provided in the active substance dossier, and the endpoints used in the risk assessment are presented in the table below.

Table 10.1-1: EU Endpoints: Toxicity of BTa ABTS-1857 to birds

Test species	Test substance	Exposure System	Endpoints used in risk assessment	Reference
Colinus virginianus (Northern bobwhite)	ABG-6305 Technical powder	Oral toxicity and pathogenicity*	LD_{50} >1714 mg BTa/kg bw/d (3.4 x 10^{11} CFU/kg bw)	IIM 8.1/01
Anas platyrhynchos (Mallard duck)	ABG-6305 Technical powder	Oral toxicity and pathogenicity*	LD_{50} >1714 mg BTa/kg bw/d (3.4 x 10^{11} CFU/kg bw)	IIM 8.1/02

* 5 days administration 30 days observation

B.9.1.2 Infectiveness to birds

No data submitted, not necessary.

B.9.1.3 Pathogenicity to birds

No data submitted, not necessary.

B.9.1.4 Risk assessment for birds

The main potential route of exposure for birds to foliar applied plant protection products is considered to be through the ingestion of residues on contaminated food, e.g. vegetation, fruit, insects and earthworms. As outlined in Vol.1, the mode of action of BTa ABTS-1857 is via the release of crystalline proteins (delta-endotoxins) under the alkaline conditions in the insect larval midgut. The pH of avian intestinal tracts is slightly acidic so even if ingestion of BTa ABTS-1857 occurs there will be no release and therefore no exposure to the active protein delta-endotoxins. Furthermore, the hydrolysed toxins have been found to bind to insect midgut cells at highly specific receptor binding sites of susceptible insects and therefore binding of toxins to avian cells is unlikely to occur. As outlined in Vol.3 B7, *B. thuringiensis* has been found to have short residues persistence on foliage, with insecticidal activity declining rapidly so that much is lost within one day, and most has gone after a period of a few days. Solar radiation was found to be a key factor in reducing the persistence of populations and the activity

of *B. thuringiensis* preparations on the leaf surface. Additionally, as outlined in Vol. 3 B8, the application of BTa ABTS 1857 to soil is not expected to significantly increase the number of *Bacillus thuringiensis* present in soil. As BT is a ubiquitous soil microbe birds are likely to be continuously exposed to low levels of BT.

No treatment related mortalities or signs of infection or pathogenicity were observed for XenTari® WG technical material (BTa ABTS-1857) in acute to short-term oral toxicity studies with both mallard duck and bobwhite quail at the highest rate tested (1714 mg/kg bw per day for five days, equivalent to approx. 3.4 x 10^{11} CFU/kg bw per day). Given the application rate of XenTari® WG is at a rate of approximately 5 x 10^{13} CFU/ha and given the short persistence on foliage as discussed above, it is unlikely that birds will be exposed to more than 3.4 x 10^{11} CFU/kg body weight per day, at which no adverse effects have been observed.

The applicant provided a risk assessment based on a calculation done according to the EFSA Journal (2009)[1] using the application rates in GAP.

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 scenarios 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 of both studies provided a LD50 of > 3.4 x 10^{11} CFU/kg b.w/d.
The density of spores in the WG formulation is 5 x10^{13} CFU/kg. The maximum of product applied is 0.002 kg/L which results in a maximum concentration in the spray liquid of 1 x 10^{11} CFU/L.

Daily dose birds:
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 9.84 x 10^{5} CFU/L for applications in fruiting vegetables, the daily dose is 4.5 x 10^{5} CFU/kg bw/d. This value is below the endpoint for birds and therefore the risk through drinking water is considered acceptable.

- **Mammals**

The acute oral LD_{50} was greater than 5050 mg/kg bw (corresponding to 9.24 x10^{10} CFU/kg b.w.). The test substance is not toxic, infective or pathogenic on the basis of the acute oral toxicity study in rats.

[1] European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12): 1438. [139 pp.].

Daily dose mammals:

The application liquid contains 1 x 10^{11} CFU/L . The daily water intake of a small granivorous mammal with a body weight of 21.7 g is 0.24 L/kg bw/d. Considering the worst PECsw of 9.84 x 10^{5} CFU/L for applications in fruiting vegetables, the daily dose is 2.36 x 10^{5} CFU/kg bw/d. This value is below the endpoint for mammals and therefore the risk through drinking water is considered acceptable.

B.9.2 Effects on aquatic organisms

B.9.2.1 Effects on fish

B.9.2.1.1 Toxicity to fish

No study on the toxicity of the representative formulation XenTari® WG on fish is available.

The ingredients of the preparation XenTari® WG are inert and are not expected to present any hazards to the environment. Therefore, studies and information on the microbial pest control agent, BTa ABTS-1857, are considered applicable and relevant with regard to the evaluation of effects on aquatic organisms of the formulated product. The aquatic toxicity of BTa ABTS-1857 was evaluated during the Annex I inclusion. No additional studies have been performed. Full details of these studies are provided in the respective EU DAR (B.9.2) and related documents and the endpoint on fish used in the risk assessment are presented in the table below.

Table 10.2-1: EU Endpoints: Toxicity of BTa ABTS-1857 to aquatic organisms

Test species	Test substance	Exposure System	Endpoints used in risk assessment	Reference
Oncorhynchus mykiss (Rainbow trout)	ABG-6305 Technical powder	96-hour	LC_{50} > 2 x 10^{7} CFU/L	IIM 8.2.1/01
		20-day	LC50 < 5.4 x 10^{10} CFU/L	IIM 8.2.1/02
		30-day	NOEC = 1.74 x 10^{10} CFU/L	IIM 8.2.1/03

* Based on a technical powder content in XenTari WG of 54%

The values presented in the table above indicate that BTa ABTS-1857 has a low acute and chronic toxicity to fish under laboratory conditions. Data are also available from a study designed to assess the infectivity and pathogenicity of BTa ABTS-1857 (ABG-6305 Technical Powder) to fish (IIM 8.2.1/02) and so this does not provide a standard toxicity endpoint for use in the risk assessment. An adverse effect on survival and growth was observed, but may have been influenced by high turbidity, suspended solids and lowered pH, resulting in fish having difficulty in identifying and consuming food particles (i.e. a physical effect).

B.9.2.1.2 Infectiveness to fish

No data available for the product. No infectivity observed in the test with the active substance.

B.9.2.1.3 Pathogenicity to fish

No data available for the product. No infectivity observed in the test with the active substance.

B.9.2.2 Effects on freshwater invertebrates

B.9.2.2.1 Toxicity to freshwater invertebrates

No study on the toxicity of the representative formulation XenTari® WG on fish is available.

The ingredients of the preparation XenTari® WG are inert and are not expected to present any hazards to the environment. Therefore, studies and information on the microbial pest control agent, BTa ABTS-1857, are considered applicable and relevant with regard to the evaluation of effects on aquatic organisms of the formulated product. The aquatic toxicity of BTa ABTS-1857 was evaluated during the Annex I inclusion. No additional studies have been performed. The endpoints used in the risk assessment are presented in the table below.

Table 10.2-1: EU Endpoints: Toxicity of BTa ABTS-1857 to aquatic organisms

Test species	Test substance	Exposure System	Endpoints used in risk assessment	Reference
Daphnia magna	ABG-6305 Technical powder	10-day	EC_{50} 2.4 x 10^8 CFU/L	IIM 8.2.2/01
		21-day	NOEC = 1.94 x 10^8 CFU/L	IIM 8.2.2/02

* Based on a technical powder content in XenTari WG of 54%

The values presented in the table above indicate that BTa ABTS-1857 shows a moderate level of toxicity to aquatic invertebrates under laboratory conditions. The chronic test was conducted over a 21-day period, as recommended in SANCO/12117/2012[2], but it is questioned whether this testing is appropriate for the testing of daphnids, due to problems with turbidity[3]. Turbidity may cause physical effects and not direct toxic effects, the former not usually being considered a “true” adverse effect in risk assessment[2]. For example, due to the turbidity caused by the particulate nature of the microbial formulation daphnid algal-feeding is reduced, and it has been observed that daphnids eventually starve if not removed from turbid conditions. It has been shown from studies on *B. thuringiensis* subsp. *israelensis* that no toxic effect is seen on daphnids in microcosm studies at the same concentrations as those used in laboratory studies. In natural environments, if water contamination with XenTari® WG were to occur, the particulate matter would rapidly sediment to the bottom of the waterway, thus alleviating any possible physical effects on daphnids. Therefore, the endpoints generated in studies ac-

[2] Working Document to the Environmental Safety Evaluation of Microbial Biocontrol Agents
[3] Report of the OECD/KEMI/EU Workshop on Microbial Pesticides: Assessment and Management of Risks. Series on Pesticides No. 76. ENV/JM/MONO(2014)2

cording to the current guidance are considered to test an extreme worst-case, and are not reflective of true toxicity, or the likely exposure scenario in the natural environment. This is considered in further detail below.

Data are also available from a study designed to assess the acute toxicity of components of BTa ABTS-1857 to daphnids (IIM 8.2.2/03). This study indicates that the toxic effects are produced by a combination of both the supernatant and the pellet and the heating reduces the supernatant toxicity to daphnids. The spore-crystal complex does not appear to contribute to the toxicity of the pellet. The exposure concentrations used in this study were in excess of those used in the 10-day acute toxicity study and so the high levels of effects seen are consistent with the EC_{50} value obtained.

Results of further toxicity testing (IIM 8.2.2/04) indicate that toxicity to daphnids was comparable between fermentation processes and between individual fermenters. Again, the exposure concentration of 70 mg/L used in this study is in excess of those used in the 10-day acute toxicity study and so the results obtained are consistent (e.g. 100% mortality seen at 34 mg/L).

Data from a published study (IIM 8.3/05) indicate that *B. thuringiensis* is rapidly phagocytosed when injected intercardially in oysters (*Crassostrea virginica)* and vegetative bacteria were rendered non-viable by intracellular digestion. These results indicate a similar course to when other bacteria deemed to be non-pathogenic to oysters were administered by parenteral injection.

B.9.2.2.2 Infectiveness to freshwater invertebrates

Not tested.

B.9.2.2.3 Pathogenicity to freshwater invertebrates

Not tested.

B.9.2.3 Effects on algae growth

Plant protection product

Reference 10.2.3/01

Reference:	M. Dorgerloh (2001) XenTari WG – Influence on the growth of the green alga, *Selenastrum capricornutum* Unpublished report, Report Nr. DOM21009
Guideline:	EEC Directive 79/831/E, EG C3, OECD 201, ISO8692
GLP:	Yes, Certified Laboratory
Material and methods:	Test substance: XenTari WG, Batch No LOT68663PG. AS content 10.3% (1.9×10^7 visible cells /mg). A static 72- hour algal growth test was used to determine the intrinsic 50% reduction in growth (EC_{50}). The intrinsic, time-independent EC_{50} values in cases of exponential growth are calculated as inhibition of growth rate (μ,r).

	Supplementary parameters, like biomass (based on area under the growth curve) and NOEC were also determined. A stock solution was prepared by adding 0.6369 g of XenTari WG to 200 mL with sterile deionised water. From this mixed solution a dilution series was made (1:1). The test organism *Selenastrum capricornutum*, strain SAG61.81 was used. Stock cultures were grown at 23 ± 2°C under 16 h light/day in cotton plugged 300 mL Erlenmeyer flasks containing 50 mL growth media. Fresh stocks were produced weekly. Pre-cultures were inoculated with 1 x 10^4 cells/mL into 200ml of a second growth media and incubated for 3 days before being used to prepare the treated and control cultures for both range finding and definitive tests. Based on the findings of initial range finding tests nominal concentrations of 10, 20, 40, 80, 160 and 320 mg/L test item were used in the definitive test. Test cultures and the cell free culture media used for quantitative analysis were prepared by mixing appropriate quantities of sterile deionised water, 10 fold concentrated nutrient solution and suitable volumes of XenTari WG stock solution. After mixing the media was divided into 2 parts, one part was used for the growth inhibition test by inoculating with 3-day old algal pre-culture to give 1 x 10^4 algal cells/mL. This medium was divided into 150 mL aliquots in the 300 mL Erlenmeyer flasks. Incubation was at 23 ± 2°C and 8000 lux 24 hours day. Sedimentation of cells and test substance was prevented by a mixer (shake cultures). *Bacillus thuringiensis* subsp. *aizawai* cell numbers were counted using a microscope at the beginning of the exposure period (two different test levels were chosen 80 and 320 mg/L). Temperature, pH and illumination were monitored. The 0-72 hour EC_{50} for biomass (E_bC_{50}) and for algal growth rate (E_rC_{50}) were calculated using probit analysis. The NOEC and LOEC were calculated by an analysis of variance. A reference chemical was used to validate the test ($K_2Cr_2O_7$).
Test substance:	Test substance: XenTari WG, Batch No: LOT68663PG. AS content 10.3% (1.9 x 10^7 visible cells /mg).
Test species:	*Selenastrum capricornutum* (current name: *Pseudokirchneriella subspicata*)
Number of test algae:	1 x 10^4 algal cells/mL (inoculum per replicate), 6 control replicates and 3 replicates per treatment concentration.
Treatments:	Nominal concentrations of 10, 20, 40, 80, 160 and 320 mg/L test item were used (equivalent to 1.9x10^8, 3.8x10^8, 7.6x10^8, 1.5x10^9, 3.0x10^9, 6.0x10^9

	CFU/L nominal)
Duration:	7 days
Test conditions:	Incubation was at 23 ± 2°C and 8000 lux 24 hours day. Sedimentation of cells and test substance was prevented by a mixer (shake cultures).
Deviations from guide-line	None
Endpoint:	pH increased for 8.3 to 9.47 during the 72-hour test, the mean temperature was 22.6°C and light intensity 7000 lux. The achieved concentrations of Bta in the 80 and 320 mg test item concentration were analytically verified to be very close to the nominal concentrations at the beginning of the test. Algal growth in the control flasks was greater than a factor of 16 after 3-days as required. A summary of the results are given in Table 1.

Table 1

Effects of XenTari WG on algal (*Selenastrum capricornutum*) growth

Biomass (0-72 h)	E_bC_{50}	119 mg/L
	LOE_bC	40.0 mg/L
	NOE_bC	20.0 mg/L
Average Growth Rate (0-72 h)	E_rC_{50}	275 mg/L
	LOE_rC	160 mg/L
	NOE_rC	80.0 mg/L

Observations:	The E_rC_{50} (0-72h) was 275 mg/L ($5.2x10^9$ CFU/L) for XenTari WG against *Selenastrum capricornutum*. The LOE_rC (0-72h) was 160 mg/L ($3.0x10^9$ CFU/L) and the NOE_rC (0-72h) was 80 mg/L ($1.5x10^9$ CFU/L). The E_bC_{50} (0-72h) was 119 mg/L ($2.26x10^9$ CFU/L), the LOE_bC (0-72h) was 40 mg/L ($7.6x10^8$ CFU/L) and the NOE_bC (0-72h) was 20 mg/L ($3.8x10^8$ CFU/L).

Results:

A summary of endpoints is given in the table below.

Table B.9.2.3: Toxic effects / Infectivity / Pathogenicity of XenTari WG to algae

Test species	*Selenastrum capricornutum* (current name: *Pseudokirchneriella subspicata*)
Toxicity of plant protection product	E_rC_{50} (0-72h): 275 mg product/L (5.2×10^9 CFU/L) E_bC_{50} (0-72h): 119 mg product/L (2.26×10^9 CFU/L)
Pathogenicity/Infecttivity	Not determined.

Comments and conclusion RMS:

The study was previously evaluated in the DAR (May 2007) and considered acceptable.

For algae the OPPTS 885.4300 guideline mainly applies. Also the OECD 201 (2006) is considered here for basic orientation, however this guideline is not entirely suitable for testing microorganisms. As the test was performed in 2001, the predecessor of this guideline (from 1984) was used. This is considered acceptable.

In the study the exposure concentrations were only verified at the beginning of the test (determined by microscopic cell counting) and it remains unclear, if the exposure remained constant throughout the test duration. No further details (e.g. wavelength) were given on the photometric methods for cell count determination and extinction in the test report. Therefore, the growth data cannot be distinguished with certainty between algal and potential bacterial growth. It is however considered very unlikely that *Bacillus thuringiensis* would proliferate in natural aquatic environments (see RAR, Vol 3, B8.1.3). In the present study bacterial growth of the test item would likely only occur as a secondary effect, where dying algae are used as a carbon source by the bacteria. Considering the high inoculation concentrations of the test item (> 1.9×10^8 CFU/L) the bacteria would have quickly outcompeted the algae (inoculated at 10^7 cells/L) , if growth conditions were suitable. Indeed, no signs of significant bacterial growth were observed in the test while at the same time algal growth was observed. Therefore, the use of the nominal exposure concentrations is considered acceptable.

The validity criteria according to OECD 201 (2006) were only partly reported. The multiplication factor of 16 was achieved, however no information on the mean CVs (coefficient of variation) for the growth rates over the complete test period (max 7%) and the for the section-by section growth rates (max 35%) in the controls was reported. Therefore the RMS calculated section-by-section CV of 15.7, 21.2 and 20.6 % for days 0-1, 1-2 and 2-3, respectively and a CV of 3.5 for the complete test duration using the double cell counts in the 6 control replicates. Hence the validity criteria as per OECD 201 are fulfilled. The test conditions (pH, temperature and light) were with acceptable according to OECD 201.

At 40 mg test item/L (7.6×10^8 CFU/L) and above concentration dependent morphological changes were observed from partly swollen algae cells after 48h over partly deformed algae cells after 48h and dissolved algae cells after 48 and 72h.

The following tables are as per original report.

Table 4. Areas ("biomass integrals") under the growth curves of *S. capricornutum* cultures treated with different concentrations of XenTari WG, and their % deviation from the control

Nominal Concentration (mg test item/L)	Areas (A) under the Growth Curves and % Inhibition					
	0 - 24 h		0 - 48 h		0 - 72h	
	A	%	A	%	A	%
Control	77	0.0	601	0.0	2642	0.0
10.0	98	-27.3	654	-8.8	2596	1.7
20.0	88	-14.3	618	-2.8	2592	1.9
40.0	68	11.7	520	13.5	2304	12.8
80.0	46	40.3	430	28.5	2072	21.6
160	58	24.7	266	55.7	724	72.6
320	22	71.4	138	77.0	334	87.4

Table 5. Growth rates in *S. capricornutum* cultures treated with different concentrations of XenTari WG, and their % deviation relative to that of control.

Nominal Concentration (mg test item/L)	Growth Rate (r) and % Inhibition					
	0 - 24 h		0 - 48 h		0 - 72 h	
	r	%	r	%	r	%
Control	1.97	0.0	1.82	0.0	1.63	0.0
10.0	2.21	-12.2	1.83	-0.8	1.61	1.3
20.0	2.09	-5.9	1.81	0.3	1.62	0.7
40.0	1.89	3.9	1.74	4.4	1.59	2.5
80.0	1.50	24.0	1.68	7.6	1.56	3.9
160	1.75	11.0	1.28	29.4	1.09	33.1
320	1.01	48.5	1.07	41.3	0.74	54.3

The RMS translated the nominal concentrations and endpoints into CFU/L:

The E_rC_{50} (0-72h) was 275 mg/L ($5.2x10^9$ CFU/L) for XenTari WG against Selenastrum capricornutum. The NOE_rC (0-72h) was 80 mg/L ($1.5x10^9$ CFU/L).

The E_bC_{50} (0-72h) was 119 mg/L ($2.26x10^9$ CFU/L), the the NOE_bC (0-72h) was 20 mg/L ($3.8x10^8$ CFU/L).

The study is considered relevant and reliable. The results and endpoints as reported above can be used in risk assessment.

B.9.2.4 Effects on plants other than algae

No further studies submitted.

B.9.2.5 Summary of the studies on aquatic organisms toxicity, infectiveness and pathogenicity

Table 9.2.5: Summary of the studies on toxicity on aquatic organisms treated with XenTari WG

Species	Test duration	Dose range	Results/ Endpoint	Observations	Reference
Toxin/Metabolite					
n.a.					
Plant protection product					
Selenastrum capricornutum	72 hours	10, 20, 40, 80, 160 and 320 mg test item/L (nominal, equivalent to $1.9x10^8$, $3.8x10^8$, $7.6x10^8$, $1.5x10^9$, $3.0x10^9$, $6.0x10^9$ CFU/L)	ErC50: 275 mg test item/L (5.2×10^9 CFU/L) ErC50: 119 mg test item/L (2.26×10^9 CFU/L)	Pathogenicity and infectivity were not studied.	Dorgerloh 2001 10.2.3/01

B.9.2.6 Risk assessment for aquatic organisms

The RMS recalculated PEDsw for eight applications in fruiting vegetables of 1.7×10^{13} CFU/ha. As drift values of 1.52% based on Rautmann drift values for seven (or more) applications on fruit crops and a TOXSWA standard ditch of 210 L/m^2 results in a PEDsw of 9.84×10^5 CFU/L. This value can be used for the ecotoxicological risk assessment.

Risk Assessment for Fish

The acute toxic endpoint for fish of 2×10^7 CFU/L and the chronic toxic endpoint of 1.74×10^{10} CFU/L are much higher than the conservative estimated PEDsw of 9.84×10^5 CFU/L. Therefore, *Bacillus thuringiensis* ssp. *aizawai* ABTS-1857 can be considered to pose a low risk to fish.

Risk Assessment for Aquatic Invertebrates

The acute toxic endpoint for aquatic invertebrates of 2.4×10^8 CFU/L and the chronic endpoint of 1.94

x 10^8 CFU/L are much higher than the conservative estimated PEDsw of 9.84 x 10^5 CFU/L. Therefore, *Bacillus thuringiensis* ssp. *aizawai* ABTS-1857 can be considered to pose a low risk to aquatic invertebrates.

Risk Assessment for Algae

The toxic endpoint for algae of 2.26 x 10^9 CFU/L is much higher than the conservative estimated PEDsw of 9.84 x 10^5 CFU/L. Therefore, *Bacillus thuringiensis* ssp. *aizawai* ABTS-1857can be considered to pose a low risk to algae.

B.9.3 Effects on bees

B.9.3.1 Toxicity to bees

Reference 10.3.1/01

Reference:	J.E. Rhodes, J. Downing (1997) Toxicological effect of XenTari WDG (ABG-6314) on a colony of honey bees
Guideline:	Pesticide Assessment Guidelines, FIFRA Subdivision M, Microbial Pest Control Agents. Hazard Evaluation: Nontarget Insects, Subsection 154A-24, US EPA Office of Pesticide Programs, 1982
GLP:	Yes (40 CFR Part 160)
Material and methods:	Test material: XenTari WDG (ABG-6314). Lot No. 12-547-PG. Bacillus thuringiensis var. aizawai (total spore count 3.55 x 10^{10} cfu/g; total microbial count 5.15 x 10^{10} cfu/g). The study was conducted in wooden nucleus hives with a single brood chamber. Each hive contained 10 frames, two being fully drawn and eight containing a sheet of comb foundation. Boardman entrance feeders, placed in the rear of each hive, offered sugar-water during acclimation and the definitive study, as forage was not readily available. A tray was placed in the bottom of each hive to serve as a dead bee trap. Bees were transferred from stock colony hives to the study hives by shaking. The feeders were filled with sugar-water and the hives were weighed the following day. Those hives below the average net weight received additional bees from the stock colonies. Five of the study hives received the remaining stock bees four days later. The study hives were located in a large circle (no information on the study area given) with all entrances facing south-east and the bees were left to acclimate for about 14 days during which time they were allowed to forage freely. In addition, the feeders were filled with sugar-water as necessary. Of the 17 hives established, 10 queen-right colonies were selected for the

	study, with five being allocated to the control and five to the test substance treatment. The study was conducted for 29 days, with test applications being made on days 0, 7, 14 and 22. The treatment rate was the maximum label rate of 2.0 lb product/acre (equivalent to 2.24 kg product/ha or 1.21 kg MPCA TP/ha). For each application, all wax surfaces were sprayed including bees brood and food stores, with any of the remaining dose aliquot (66 mL per hive) being sprayed on top of the frames. The amount of treatment solution applied was calculated on the basis of a total super area per hive of 10 ft^2 (i.e. the frames received the equivalent field rate). The test solutions were prepared on the morning of each application and were prepared in sugar : water solution (1 : 1), with the control receiving sugar-water solution only. The activity of the test substance in water was checked prior to study initiation, at termination and on day 7 (sugar-water): the mean number of colonies obtained were 1.25 x 10^{10}, 1.5 x 10^{10} and 1.6 x 10^{10} cfu/g, respectively. Dead bees were collected from the traps daily and counted. Mark/re-capture trials to check trap efficiency were conducted in days 5, 9, 10, 16, 23 and 26. Three hives were randomly selected for each trial on days 5, 9, 10 and 23, five hives were selected on day 16 and four hives were selected on day 26. Twenty marked dead bees were placed in the top of the hive and the traps checked once or twice a day and the number of marked bees was recorded until no more were recovered. Trap efficiency varied between 15 and 85% (mean 61%). The mean weight of the bees was determined before treatment by weighing 15 bees from each hive and again on day 28 (25 bees per hive). The bee population was determined at the beginning of acclimation by weighing the study hives with and without bees. The population was calculated at the end of the study (day 29), again by weighing the hives with and without bees. The empty hive weights at the beginning and end of the study were also used to evaluate hive production (honey, brood, nectar, pollen and wax). Test conditions were temperature: 2 to 26°C; humidity – 32 to 98%; rainfall – trace to 33 mm; lighting cycle – natural daylight.
Test substance:	XenTari WDG (ABG-6314). Lot No. 12-547-PG. Bacillus thuringiensis var. aizawai (total spore count 3.55 x 10^{10} cfu/g; total microbial count 5.15 x 10^{10} cfu/g).
Test species:	Apis mellifera
Number of test animals:	10 queen-right colonies were selected for the study, with five being allocated to the control and five to the test substance treatment.
Treatments:	For each application, all wax surfaces were sprayed including bees brood

	and food stores, with any of the remaining dose aliquot (66 mL per hive) being sprayed on top of the frames.
Duration:	The study was conducted for 29 days, with test applications being made on days 0, 7, 14 and 22.
Test conditions:	Test conditions were temperature: 2 to 26^{o}C; humidity – 32 to 98%; rainfall – trace to 33 mm; lighting cycle – natural daylight.
Deviations from guide-line	None
Endpoint:	Adult mortality: There were no statistically significant effects observed between the control and test substance treatment groups ($p < 0.05$). The weekly and cumulative adult mortality is summarised in Table 1. Population change: colony observations showed that all colonies were queen-right and active (building comb, raising brood and actively foraging). All test colonies showed natural population attrition over the course of the study (effects of aging in the initial mixed-age distribution, together with the lack of initial brood at test start). The mean population decrease was 13,997 bees in the control and 16,766 bees in the XenTari treatment (68.1 and 70.4% decrease, respectively). No statistically significant differences in population change were observed between the control and treated groups ($p < 0.05$). The population changes are summarised in Table 2. Change in hive weight: On day 0 the control hives had a mean weight of 12.0 kg and the XenTari treated hives had a mean weight of 12.2 kg. On day 29 the mean weights were 14.5 and 14.2 kg, respectively resulting in percentage increases of 21.2 and 16.6%, respectively. The final weights were subject to a high level of variability reflecting the ageing populations and the natural field conditions together with experimental aspects of the study. Thus, one control hive showed a weight loss that was attributed to comb removal in order to encourage use of the frames provided. The hive weights are summarised in Table 3. Visual observations and queen survival: the hives were visually inspected once per week with observations of the amount of honey and comb produced and any effects on colony behaviour. These indicated that all colonies were growing, foraging and producing wax, honey and brood in a normal manner. Queen survival was 100%.

Table 1

Weekly and cumulative adult honey bee mortality (mean ± SD)

Treatment	Week 1	Week 2	Week 3	Week 4 (day 29)	Total
Control	176 ± 197	141 ± 116	214 ± 107	83 ± 71	615 ± 466
XenTari (2.24 kg a.s./ha)	142 ± 235	116 ± 132	119 ± 88	39 ± 19	416 ± 442

Table 2

Number of bees present in hives at test initiation and termination (mean ± SD)

Treatment	Pre-application	Termination (day 29)	% change
Control	20,553 ± 2909	6556 ± 1858	-68.1 ± 8.1
XenTari (2.24 kg a.s./ha)	23,037 ± 6505	6271 ± 1505	-70.4 ± 12

Table 3

Hive weight (bees, honey and wax) at test initiation and termination (mean ± SD)

Treatment	Initiation (day 0)	Termination (day 29)	% change
Control	12.0 ± 0.39 kg	14.5 ± 2.3 kg	21.2 ± 19
XenTari (2.24 kg a.s./ha)	12.2 ± 0.62 kg	14.2 ± 1.6 kg	16.6 ± 15

Observations:	A 29-day study was conducted with XenTari WDG (ABG-6314) to evaluate effects on honey bee survival and colony performance (adult bee population, hive weight and behaviour). Four applications were made at weekly intervals (days 0, 7, 14 and 22) directly into the hive at a rate equivalent to 1.21 kg MPCA TP/ha. Effects were compared to control hives treated with a sugar-water solution only. The results of the study indicate that the control and treated hives responded in a similar manner over the 29-day duration of the study and there were no significant effects between them for any of the parameters assessed. It was concluded that the test rate of 2.24 kg product/ha (1.21 kg MPCA TP/ha), applied four times at weekly intervals was a no-observable effect level (NOEL).

Results:

A summary of endpoints is given in the table below.

Table B.9.3.1.a: Toxicity effects/infectivity/pathogenicity of the MPCA to bees

Test species	Apis mellifera
Toxicity	Not toxic to honeybees colonies up to 2.24 kg product/ha (i.e. 7.95 x 10^{13} CFU/ha), exposure through overspray.
Infectivity / Pathogenicity	Not studied.

Comments and conclusion RMS:

The effect of XenTari (3.55 x 10^{10} CFU/g) on the colony survival of honeybees was investigated by spraying 5 colonies of bees with the equivalent of 2.24 kg product/ha. The concentration of the CFUs in product is similar to the current product while the application rate is 2 times higher than that of the current product. The spraying of the colonies was on day 0, day 7, day 14 and day 22. The test continued until day 29. Another five colonies served as control. The product in sugar water was sprayed on the wax surface, bee brood, food stores and on the top frames. The concentrations of the active substance in the sugar water was determined at the initiation and termination of the test and on day 7 and were 1.25 x 10^{10} CFU/g, 1.5 x 10^{10} CFU/g and 1.6 x 10^{10} CFU/g, respectively.

The quantitative parameters determined were: adult mortalities, population change (queen-right and activity) and change in hive weight. There were no statistical significant differences between the controls and the treatments for any of these parameters. Visual observations included the effects on honey and comb produced, effects on colony behavior and queen survival. None of these parameters were affected.

It is noted that the study was performed in October, when beehives have started their natural decline to prepare for overwintering. The effects may be different on growing colonies.

RMS notes that the exposure of the hives was not according to the EPPO guideline. The current design included the direct spray of the hives while the EPPO guideline recommends the exposure of the honey bee colonies to the treated fields with full flowering crops. After 7 days the colonies are removed to areas with no main attractive crops. Although it can be argued that an overexposure occurred in the current test, the oral exposure of bees through pollen and nectar cannot be guaranteed. Furthermore, the effects on foraging activity cannot be assessed.

It can be concluded that under the conditions of this test there were no effects on adults mortality, queen survival and bee behaviour.

The study can be used in the risk assessment.

Reference10.3.1/02

Reference:	K. Bolckmans (1995) Toxicity of the biological insecticide ABG-6314 for bumblebees (Bombus terrestris L.). BIOBEST N.V. Unpublished report No.: 9505151F
Guideline:	None
GLP:	No
Material and methods:	ABG-6314 was tested for its toxicity to adult bumblebees (Bombus terrestris) under standardised laboratory conditions. For each treatment, there were two groups of 10 bumblebees of approximately equal size obtained from artificially reared colonies, each housed in wooden cages (115 x 115 x 80 mm) with a metal mesh bottom and a transparent plastic lid. There were five tests: oral toxicity via individual feeding; oral toxicity via group feeding; indirect contact toxicity; topical (direct overspray) toxicity and inhalation toxicity. In the oral tests, the test solutions were made up in 50% sucrose solution. For individual feeding, the bees were starved for two hours and placed in transparent plastic tubes and fed 30 μL of test solution in a 1 mL plastic micropipette, which was inserted through a hole in the lid of the tube. After the bees had taken all the administered test solution they were placed in groups of 10 in the test cages and fed 50% sucrose solution. In the group feeding test, cages of 10 bees were fed with the test solution for 24 hours and then supplied 50% sucrose solution for the remainder of the test. In the contact toxicity test, a piece of absorbent paper drenched with the test solution (in acetone) was placed in the bottom of each test cage. In the topical toxicity test, batches of 10 bees were anaesthetised with CO_2 and then directly sprayed with 1 mL of the test solution (in water). In the inhalation toxicity test, the test cages were placed above a Petri dish containing the test solutions (in water) so that any vapour could enter through the wire mesh bottom. For each test there were four treatments: ABG-6314 applied at 0.1 and 0.2%; a toxic standard (oral toxicity – dimethoate; topical, inhalation and contact toxicity – ethyl parathion); a control using the carrier only (oral toxicity – 1/1 sucrose solution; topical and inhalation toxicity – water; contact toxicity – acetone). The test cages were placed in controlled climate rooms at 29oC and 60% relative humidity and the bees were fed 50% sucrose solution. Assessments for mortality and abnormal behaviour were carried out at 30 and 45 minutes then 1, 2, 4, 24, 48 and 72 hours after dosing.
Micro-organism	Biological insecticide ABG 6314 (Bt subsp. aizawai)
Test species:	Bumble-bee (Bombus terrestris)

Number of test animals:	10 bumble-bees x 2 replicates/dose
Treatments:	Oral toxicity: 0.1-0.2 % solution; Topical toxicity: 0.1-0.2% solution; Tarsal toxicity: 0.2-0.4% solution; Inhalation toxicity: 0.2-0.4% solution
Duration:	Observations were done at 30', 45', 1h, 2h, 4h, 24h, 72h
Test conditions:	29 °C and 60% RH; dark
Deviations from guide-line	-
Endpoint:	The results are summarized in Table 1. There was no ABG-6314 treatment-related mortality in any of the tests and no abnormal changes in the behaviour of the bumblebees was observed.

Table 1

Bumble bee mortality (number of bees) following 72 hours exposure to ABG-6314

Test	ABG-6314				Toxic standard		Control	
	0.1%		0.2%					
Oral	0	0	0	1	10	10	0	0
Group oral	0	0	1	0	10	10	0	0
Contact (indirect)	0	0	0	1	10	10	1	0
Contact (topical)	0	0	0	0	10	10	0	0
Inhala-tion	0	0	0	0	10	10	0	0

Observations:	The results of this study indicates that ABG-6314 (Xentari WDG) is non-toxic to bumblebees (Bombus terrestris) when exposed at concentrations of up to 0.2% via direct (overspray) contact, indirect contact, oral (individual and group feeding) and inhalation routes of exposure.

Comment (DAR 2007): The experiment was performed for a short period (3 days) and under dark conditions, in which trophic activity of insects is highly reduced. No GLP and guideline in the experiment

Results:

A summary of endpoints is given in the table below.

Table B.9.3.1: Toxic effects / Infectivity / Pathogenicity of XenTari to bumblebees

Test species	*Bombus terrestris*
Toxicity of plant protection product	Test solutions in % ABG-6314 in 50% sucrose solution 72h Oral (individual feeding): no mortality at 0,2% (equals 2g product/L, 1x 10^{11} CFU/L, 3x10^{6} CFU/bee, 900 IU/bee) 72h Oral (group feeding): no mortality at 0,2 % (equals 2g product/L and 1x 10^{11} CFU/L, 3x10^{6} CFU/bee, 900 IU/bee) 72 Contact (topical overspray): no mortality at 0,4% (equals 4 g product/L and 2x10^{11} CFU/L) (in all cases the highest dose tested)
Pathogenicity/ Infectivity	Not studied.

Comments and conclusion RMS:

The study was previously evaluated in the DAR from 2007 and the comment above was made.
Although no guideline is directly applicable to the testing of microorganisms in bumble bees, the current OECD draft guidelines from 2016 for testing of the effects on bumblebees of acute exposure to chemical substances were taken into consideration.

The study was not carried out under GLP and the report suffers from a few additional shortcomings. The origin and the stage of the colony are not mentioned. The selection procedure of the test animals was not reported and it is not clear, if they were equal in weight. It is not clear, if the bumble bees originated from more than one colony. The amount of acetone used for tarsal toxicity is not reported (required to not exceed 5%). In the tables from p5ff exposures of 0.2 and 0.4% are mentioned for topical and for inhalation toxicity, whereas in the text only exposure up to 0,2% is mentioned. Therefore it is not clear what the exposure in the topical toxicity test was.

The exact nature (species) / origin and composition (purity or activity) of the test substance is not mentioned in the report. However, Volume 4 lists ABG-6314 as the XenTari WG formulation and informs about the composition: ABG-6314 contains 54% (w/w) technical powder, 15000 IU/mg, and according to the GAP a spore count of 5 x 10^{13} CFU/kg. Therefore, the information of exposure (0.1, 0.2 or 0.4 % ABG-6314 in 50% sucrose solution) is translated into units more useful for risk assessment:

Exposure in 50% sucrose solution						Exposure/bee (oral)	
%	interpreted as	g prod/L	g a.s./L	CFU/L	IU/L	CFU/bee	IU/bee
0,1	0,1g product/100mL	1	0,54	5x10^{10}	1.5x10^{7}	1.5x10^{6}	450
0,2	0,2g product/100mL	2	1,08	1x10^{11}	3x10^{7}	3x10^{6}	900
0,4	0,4g product/100mL	4	2,16	2x10^{11}	6x10^{7}	N/A	N/A

The GAP reports that maximum 0.1 kg formulated product/hL is used in the field. This translates into 1 g product/L spray solution. Therefore the results from this study are relevant to the uses assessed.

The guidance advises to keep bumble bees individually in cages to avoid hierarchy fights and the stress resulting from it. In the present study only the individual feeding test was done under single housing conditions. As no control mortality and no effects of the test substance occurred this is however not considered problematic in this specific case.
The methods for contact exposure in this test were tarsal exposure (bumblebees walking on exposed paper) and direct overspray. It is noted that both are different from the standard method recommended in the draft guideline, i.e. application of a droplet on the thorax.
The tests were neither designed as a dose-response, nor as a limit test (per test two ABG-6314 treatments, one toxic reference and one control were carried out with 10 bumble bees per replicate and two replicates for each treatment). Therefore the requirements for replication (30 single bumble bees for a dose response design and 50 for a limit test design) are not fulfilled. No LD50 values were calculated, but from the data it could be derived that the LD50 would be above 0.4% (contact toxicity) and above 0.2% (oral toxicity).
The validity criteria of the draft guidelines for bumble bees (< 10% mortality in the controls and ≥ 50% mortality at the end of the test in the toxic reference) are fulfilled. The concentration of the positive control dimethoate was 31.2 ug/bee and was high enough to cause mortality.

According to the OECD draft guidelines the 72h test duration of this study was sufficient. However, usually for the testing of microorganisms longer test durations are required according to OPPTS guidelines, certainly, if pathogenicity is investigated. Therefore the results cannot be used for any conclusions on pathogenicity. Infectivity was not studied.

The results from this study on oral and contact toxicity can be used in risk assessment.

Reference 10.3.1/04

Reference:	Mommaerts, V., Jans, K., Smagghe, G. (2009). Impact of *Bacillus thuringiensis* strains on survival, reproduction and foraging behaviour in bumblebees (*Bombus terrestris*). Pest Management Science, 66, 520-525
Guideline:	Not specified
GLP:	No
Material and methods:	The objectives of the present research were to evaluate the side effects of two Bt products, Dipel (Btk) and Xentari (Bta), on the bumblebee *Bombus terrestris* (L.). Workers were exposed in microcolonies of each 5 worker bees

	in the laboratory to the Bt products at their respective field recommended concentration through dermal contact and orally via feeding treated sugar water and pollen. For the contact applications, 50 µL of the aqueous concentration was topically applied on the dorsal thorax of each worker with a micropipette. For the oral treatments, the nests were exposed *ad libitum* for 11 weeks to 500 mL of sugar water with the product or to pollen saturated the prepared recommended field rate concentration. Observations of dead worker bees, male offspring in nests was done on a weekly basis. The impact on foraging behaviour was tested as well by connecting two artificial nest boxes with a tube. In one box, five newly emerged workers constructed their nest. When third- and fourth-instar larvae appeared in the nest; food was removed from this box and placed in the second box. Only the experiment with Xentari (Bta) is presented here.
Micro-organism	*Bacillus thuringiensis* subsp. *aizawai* (in Xentari)
Test species:	*Bombus terrestris*
Number of test animals:	20 worker bumble bees per treatment, in total 100 bumble bees (excluding the experiment on foraging behaviour)
Treatments:	Microconlonies with 5 workers each, in replicates of 4 for each treatment route, 3 treatment routes: dermal contact, oral via treated sugar water, oral via treated pollen. 1 negative control (only water) and 1 positive control (Confidor, immidachloprid at recommended field concentration (0.1% in water). Experiment performed twice independently. Treatment was the recommended field rate of the product (0.1%) and a 1/10 dilution of the recommended field rate for Xentari (0.01%) in sugar water was additional tested tested due to the high mortality in the 0.1% treatment. Xentari WG contained 1.5 x10^4 IU/mg and its recommended field rate was 0.1% formulation in water, leading to a recommended field rate of 1.5 x 10^4 IU/mL.
Duration:	11 weeks
Test conditions:	28-30°C, 60-70% humidity, continuous darkness
Deviations from guideline	Not applicable.

Endpoint:	Survival: number of suviving worker bees, Reproduction: number dead larvae and number of drones Foraging behaviour: effects on reproduction and larvae survival were measured
Observations:	Xentari at its recommended field rate of 1.5x 10^4 IU/mL of 0.1% resulted in 100% acute worker mortality when delivered orally via treated sugar water. An additional test with a lower dose of 0.01% (1/10 of the MFRC) resulted in 0% mortality of the treated worker bees. Exposure to treated pollen at 0.1% did not lead to an increased mortality of workers. Sublethal effects (loss of reproduction) of 100% were observed for the recommended field rate treatment (0.1%) when delivered via sugar water, while delivery via pollen resulted in 31% reduction in reproduction. Dermal contact exposure did not lead to a reduction in reproductive capability. No negative effects on reproduction with the lower dose (0.01%) were indicated, as there were no differences between the mean number of drones in the treated and control group after 11 weeks. In the foraging behaviour test, no negative effects on microcolony performance were observed. Conclusions: The results with Bt subsp. *aizawai* demonstrated that, in general, the Bt strains are safe to *B. terrestris* bumblebees.

Results:

A summary of endpoints is given in the table below.

Table B.9.3.1.b: Toxic effects of Xentari to bumblebees

Test species	*Bombus terrestris* (Bumble bee)
Toxicity	100% mortality orally via sugar water at 1.5 x 10^7 IU/L (5x10^{10} CFU/L) 0% mortality orally via sugar water at 1.5 x 10^6 IU/L (5x10^9 CFU/L) 100% reduction in reproduction orally via sugar water at 1.5 x 10^7 IU/L (5x10^{10} CFU/L) 0% reduction in reproduction orally via sugar water at 1.5 x 10^6 IU/L (5x10^9 CFU/L) 31% reduction in reproduction orally via pollen at 1.5 x 10^7 IU/L (5x10^{10} CFU/L)
Infectivity / Pathogenicity	Not tested.

Comments and conclusion RMS:

This scientific paper did not apply the standards of any guideline or GLP. While the quality of the scientific study itself is acceptable and provides valuable supporting information, the outcome of the study itself is difficult to translate into appropriate use for regulatory purposes. While the used product

have been identified through the trade names, the exact strains, the manufacturers, the source and the Lot or Batch numbers were not reported. Equally the activity of the product was only reported in IU/mg formulated product which refers to the activity in terms of Bt toxin.

It is clear that chronic exposure over 11 days at the recommended field rate of Xentari lead to significant mortality and reproductive effects when administered via sugar water and to a lesser extent via pollen.

The tabulated information on sublethal effects as presented by the study authors is included below.

Table 2. Sublethal effect on the reproduction of workers of *Bombus terrestris* by two *Bt* products, Dipel® and Xentari®, when treated at their respective MFRC or at a 1/10 dilution of the MFRC via contact exposure and via oral exposure via feeding treated sugar water and treated pollen over a period of 11 weeks[a,b]

Product	Contact	Sugar water	Pollen
Dipel® (0.1%)	25.6 (± 1.4) a	33.5 (± 1.5) a	29.5 (± 3.5) a
Xentari® (0.1%)	30.5 (± 1.0) a	0.0 (± 0.0) b	19.8 (± 1.3) b
Xentari® (0.01%)	ND	33.5 (± 3.3) a	ND
Control	30.8 (± 3.0) a	28.9 (± 1.1) a	28.8 (± 2.0) a

[a] The data are expressed as mean numbers of drones per nest (± SEM) based on four artificial nests per treatment and five workers per nest, and the experiment was 2 times independently repeated. ANOVA resulted in one group for contact exposure ($F = 1.595$; df = 23; $P = 0.227$), in two groups for sugar water exposure ($F = 63.128$; df = 31; $P < 0.001$) and in two groups for pollen exposure ($F = 7.459$; df = 23; $P = 0.004$). Values per column that are followed by a different lower-case letter are significantly different (*post hoc* Tukey–Kramer test with $P = 0.05$).
[b] ND = not determined.

Table 3. Sublethal effects of the two *Bt* products, Dipel® and Xentari®, on nest performance[a] including the worker foraging behaviour of *Bombus terrestris*. Worker bees were orally exposed to sublethal concentrations of each *Bt* product via feeding treated sugar water over a period of 11 weeks

Product	Mean numbers of dead larvae per nest (± SEM)	Mean numbers of drones per nest (± SEM)
Dipel® (0.1%)	10.9 (± 0.9) ab	28.6 (± 3.5) a
Xentari® (0.01%)	17.3 (± 10.0) a	33.8 (± 1.5) a
Control	12.3 (± 1.0) a	28.4 (± 2.9) a
Imidacloprid (0.00001%)	0.0 (± 0.0) b	5.6 (± 0.1) b

[a] As measures of nest performance, the data are expressed as mean numbers of dead larvae and live drones per nest (± SEM) based on four artificial nests per treatment and five workers per nest each, and the experiment was 2 times independently repeated. ANOVA resulted in two groups for larval mortality ($F = 40.657$; df = 31; $P < 0.001$) and two groups for drone production ($F = 5.768$; df = 31; $P = 0.003$). Values per column that are followed by a different lower-case letter are significantly different (*post hoc* Tukey–Kramer test with $P = 0.05$).

Exposure to 0.1% Xentari (i.e. 5 x 10^{10} CFU/L, 1.5 x 10^{7} IU/L) of the bumblebees via treated pollen resulted in a significant reduction of 31% in reproduction.

No effects on foraging behaviour and nest performance were detected at the 0.1% field application rates of 5 x 10^{10} CFU/L, 1.5 x 10^{7} IU/L.

The high toxicity of Xentari to bumblebees when exposed via sugar water but low toxicity when exposed to the same dose in treated pollen was attributed to the feeding preferences of bumblebees as it was demonstrated that they prefer nectar over pollen. It is implied, though not measured, that the bumblebees in the test did not feed as much on the treated pollen than on the treated sugar water.

The high toxicity of Xentari as compared to Dipel (results not reported here) was possibly attributed to the Cry 1C and Cry 1D proteins present in Xentari but not in Dipel. It was hypothesized that the activity of these toxins in the bumblebees might indicate not yet discovered binding receptor/sites.

Regarding the sublethal effects and considering the information available in the peer-reviewed literature, the authors suggest that susceptibility to Cry toxins might vary between taxa and therefore they recommend that the risk assessment studies should be tailored on species level with realistic field doses.

The authors further conclude that the detrimental effects depend on the strain and the route of exposure.

The study is acceptable and the results can be used in the risk assessment.

B.9.3.2 Infectiveness to bees

Not tested.

B.9.3.3 Pathogenicity to bees

Not tested.

B.9.3.4 Summary and risk assessment for bees

Table 9.2.5: Summary of the studies on toxicity on aquatic organisms treated with toxin/metabolite from the active ingredient or the plant protection product.

Species	Test duration	Dose range	Results/ Endpoint	Observations	Reference
Toxin/Metabolite					
n.a.					
Plant protection product					
Apis mellifera colonies	29 days	2.24 kg product/ha (7.95×10^{13} CFU/ha)	LD50 > 2.24 kg product/ha (7.95×10^{13} CFU/ha)	Pathogenicity and infectivity were not studied.	Rhodes 1997 10.3.1/01
Bombus terrestris	72 hours	0.1%: 1 g product/L, $1.5x10^{7}$ IU/L, $5x10^{10}$ CFU/L) 0.2%: 2g product/L, $3x10^{7}$ IU/L, $1x10^{11}$ CFU/L 0.4%: 4g product/L, $6x10^{7}$ IU/L, $2x10^{11}$ CFU/L)	Test solutions in % ABG-6314 in 50% sucrose solution 72h Oral (individual feeding): no mortality at 0,2% (equals 2g product/L, $1x\ 10^{11}$ CFU/L, $3x10^{6}$ CFU/bee, 900 IU/bee) 72h Oral (group feeding): no mortality at 0,2 % (equals 2g product/L and $1x\ 10^{11}$ CFU/L, $3x10^{6}$ CFU/bee, 900 IU/bee) 72 Contact (topical overspray): no mortality at	Pathogenicity and infectivity were not studied.	Bolckmans 1995 10.3.1/02

Species	Test duration	Dose range	Results/ Endpoint	Observations	Reference
			0,4% (equals 4 g product/L and $2x10^{11}$ CFU/L) (in all cases the highest dose tested)		
Bumble bee *(Bombus terrestris)*	Oral and contact Exposure (11 weeks)	*Bta* (Xentari)	100% mortality orally via sugar water at 1.5×10^4 IU/mL 0% mortality orally via sugar water at 1.5×10^3 IU/mL 100% reduction in reproduction orally via sugar water at 1.5×10^4 IU/mL 0% reduction in reproduction orally via sugar water at 1.5×10^3 IU/mL 31% reduction in reproduction orally via pollen at 1.5×10^4 IU/mL. No effects via dermal exposure.		Mommaerts, V., Jans, K., Smagghe, G. (2009)
Bumble bee *(Bombus terrestris)*	Oral and contact Exposure (11 weeks)	*Btk* (Dipel)	3% mortality via contact at 1.6×10^4 IU/mL		Mommaerts, V., Jans, K., Smagghe, G. (2009)

Species	Test duration	Dose range	Results/ Endpoint	Observations	Reference
			5% mortality via sugar water at 1.6 $x10^4$ IU/mL 0% mortality via treated pollen at 1.6 $x10^4$ IU/mL No effects on reproduction at 1.6 $x10^4$ IU/mL		

Risk assessment product

The applicant claims for outdoor and indoor uses a maximum intended use rate of Xentari WG of 0.5 - 1 kg product/ha (i.e. 0.270 - 0.540 kg a.s./ha). Considering the max application rate of 1kg product/ha and a water volume of 500 -1000 L/ha, the application is 2 g – 1 g product/L. Considering the concentration of 15000 IU/mg product, this translates to 3 x 10^7 IU/L or 3 x 10^4IU/mL and 1.5 x 10^7 IU/L or 1.5 x 10^4IU/mL. Considering the min application rate of 0.5 kg product/ha and a water volume of 500 - 1000 L/ha, the application is 1 g – 0.5 g product/L. Considering the concentration of 15000 IU/mg product, this translates to 1.5 x 10^7 IU/L or 1.5 x 10^4IU/mL and 7.5 x 10^6 IU/L or 7.5 x 10^3 IU/mL.

There were no effects on honeybee colonies at applications two times higher than the max field application rate of 1 kg product/ha.

No mortality to bumblebees was observed through the acute oral and contact exposure at rates equivalent to maximum field application rate and two times higher the field application rate, respectively.

In the study by Mommaerts et al. (2009), 100% mortality and as well 100% reduction in reproduction via sugar water was observed at 1.5 x 10^4IU/mL a dose similar to the current field application rates. The current range of application is 7.5 x 10^3 IU/mL to 3 x 10^4IU/mL . Therefore similar effects can be expected for the current applications. The RMS is of opinion that if no further information is provided a restriction sentence and a warning sentence are necessary prohibiting the application of the product when the crop is flowering

Further infectivity and pathogenicity must be addressed.

Risk assessment active substance

In the oral test the 7 day LD50 was set at > 2 x 10^{11} CFU/L. the current product is applied at 5 x 10^{13} CFU/ha and considering a water volume of 500 to 1000 L/ha, the application rate are in the range of 1 x 10^{11} CFU/L and 5 x 10^{10} CFU/L. Therefore the effects seen in the 7 day study are at doses 2 to 4

times higher than the field application rates. No effects on honeybee larvae were observed at concentrations in diet higher than the current maximum field application rate. Based on these results, no risk to honey bees and honey bee larvae is expected through oral exposure to the active substance.

B.9.4 Effects on arthropods other than bees

B.9.4.1 Toxicity to arthropods other than bees

Plant protection product

Reference 10.4/01

Reference:	M. Schuld (2002) XenTari WG: Toxicity test on the egg parasitoid, Trichogramma cacoeciae Marchal (Hymenoptera, Trichogrammatidae) under laboratory conditions. Unpublished report 20011423/01-NLTc
Guideline:	Hassan et al., (2000): A laboratory method to evaluate the side effects of plant protection products on Trichogramma cacoeciae (Hymenoptera, Trichogrammatidae); IOBC Guidelines to evaluate side effects of plant protection products to non-target arthropods; Dreier-Druck, Germany.
GLP:	Yes (certified laboratory)
Material and methods:	Test material: XenTari WG. Lot No 77-021-PG. Bacillus thuringiensis var. aizawai nominal content: 10.3% w/w (meets label claim of 35,000 Diamondback Moth Units/mg). This study evaluated the effects of XenTari WG (a.s. Bacillus thuringiensis var. aizawai) on the lepidopteran egg parasitoid Trichogramma cacoeciae. The test involved exposing adult wasps aged about 24 hours to freshly dried residues of the test substance applied to an inert substrate (glass plates), with four replicates per treatment. The test substance was applied at an application rate of 33.40 kg product/ha (equivalent to 3.44 kg a.s./ha in terms of parasporal crystals or 18.036 kg MPCA TP/ha). Perfekthion EC (dimethoate, purity 395.7 g/L) was used as a toxic standard at an application rate of 0.34 g a.s./L and deionised water was applied as a control (200 L/ha). All treatments were applied in a spray volume of 200 L water/ha using a calibrated automatic laboratory spraying-cabin. The treatments were applied to glass plates and when the spray deposits had dried they were assembled into the test cages using an aluminium frame and the adults introduced. After 24 hours exposure, the surviving adults were offered host eggs of Sitotroga cerealella (the eggs were glued onto card in three circles of about 500 eggs each). The control and test XenTari treatment were offered excess host eggs on days +1 (4500 eggs), +2 (3000 eggs) and +4 (1500 eggs) after the start of exposure. All egg cards were removed on day +7 and incubated

	in glass tubes for 2 to 5 days until evaluation (if assessment took place after this the eggs were stored in a refrigerator to stop further development). An assessment was made of the number of parasitised eggs (identified by their black colour). The original adult population in each exposure cage was determined by an investigation of the hatching tubes used to introduce the adults into the test cages. After further incubation the number of remaining adults was counted together with the number of parasitised eggs, the difference between them being the number introduced as starting population. During the exposure period honey-gelatine was offered as food. The exposure and subsequent parasitisation phase took place under the following conditions: 22.5 to 26.0oC; 68 to 81% relative humidity and 16 h light (2521 lux) : 8 hours darkness.
Test substance	Test material: XenTari WG. Lot No 77-021-PG. Bacillus thuringiensis var. aizawai nominal content: 10.3% w/w
Test species:	Trichogramma cacoeciae Marchal (Hymenoptera, Trichogrammatidae)
Number of test animals:	4 replicates
Treatments:	3.44 kg a.s./ha
Duration:	7 days
Test conditions:	22- 26 ^{o}C; 68 to 81% relative humidity and 16 : 8 L:D
Deviations from guideline	None
Endpoint:	The mean number of parasitised eggs in the control and XenTari treatment were similar (17.44 and 17.71 eggs/female, respectively). The number in the toxic standard showed a marked reduction compared to the control that was statistically significant. The test was considered valid as the mean number of parasitised eggs per female in the control was ≥15 and the reduction in parasitism in the toxic standard was >50%. The results are presented in Table 1.

Table 1

Mean number of parasitised eggs per female exposed to treated glass plates

Treatment	Control	XenTari WG (3.44 kg a.s./ha)	Toxic standard (0.34 g a.s./ha)
Mean no. of eggs[1]	17.44	17.71	0.10[3]
S.D.[1]	3.95	2.72	0.11
R (%)[2]	-	101.55	0.57

1 Mean number of parasitised eggs/female (4 replicates); S.D. – standard deviation

[2] Reproduction rate compared to the control

[3] Significantly different from the control (Dunnett's Test; p <0.05)

Observations:	XenTari WG caused no adverse effects on adult Trichogramma cacoeciae when exposed to fresh, dried residues at a rate of 3.44 kg a.s./ha (parasporal crystal) or 18.036 kg MPCA TP/ha. The reproduction rate for the test substance was calculated to be 101.55% relative to the control. A statistically significant difference compared to the control was only found in the toxic standard (dimethoate applied at 0.34 g a.s./ha), where the relative reproduction rate was 0.57%. Together with the reproduction rate in the control (mean of 17.44 parasitised eggs/female), this indicates that the test was valid.

Results:

A summary of endpoints is given in the table below.

Table B.9.4.1: Toxic effects / Infectivity / Pathogenicity of XenTari WG to arthropods other than bees

Test species	*Trichogramma cacoeciae*
Toxicity of plant protection product	Only sublethal effects were studied: Reproduction and Parasitization: ER50 > 33.4 kg product/ha (equals 3.44 kg a.s./ha)
Pathogenicity / Infectivity	Not studied.

Comments and conclusion RMS:

The study was previously evaluated in the DAR of 2007 and considered acceptable.

The current applicable OPPTS 885.340 (1996) guideline was considered for a re-evaluation of the study. The IOBC guideline used in the present study is however considered relevant as well, as it is specific to the organism tested.

The RMS maintains the conclusions from the DAR (2007) and considers the study acceptable and

suitable for use in risk assessment.

B.9.4.2 Infectiveness to arthropods other than bees

Not investigated.

B.9.4.3 Pathogenicity to arthropods other than bees

Not pathogenicity observed.

B.9.4.4 Summary and risk assessment for non-target arthropod species other than bees

Species	**Test duration**	**Results/ Endpoint CFU/L**	**Exposure CFU/L**	**MoS**
Active substance				
Metaseiulus occidentalis (predatory mite, predator)	max 8 days	ER50 mortality: > 4.8 x 10^{12}	1 x 10^{11}	48
Tetranychus urticae (spider mite, prey)		ER50 mortality adults < 4.8 x 10^{12} ER50 survival protonymphs > 4.8 x10^{11} - < 4.8 x10^{12}		<48 > 4.8 - < 48
Trichogramma pretiosum	max 10 days	ER50 mortality: > 4.8 x 10^{12} CFU/L		> 48
Ladybird beetle *Hippodamia convergens*	27 days	Dietary ER50 mortality: > 4.69x10^{11}		> 4.7
Green lacewing *Chysoperla carnea*	7 days	ER50 mortality: > 4.8 x 10^{11} while < 4.8 x		> 4.8 - < 48

Species	**Test duration**	**Results/ Endpoint CFU/L**	**Exposure CFU/L**	**MoS**
		10^{12} ER50 reproduction (pupation success): > 4.8 x 10^{11}		
Species	**Test duration**	**Results/ Endpoint Kg prod-uct/ha**	**Exposure Kg prod-uct/ha**	**MoS**
Trichogramma cacoeciae	7 days	> 33.4	1	33.4

The active substance and the product do not have unacceptable effects on the arthropods used in the IPM such as *Metaseiulus occidentalis*, *Hippodamia convergens*, *Chrysoperla carnea*, *Trichogramma* spp. No effects on the adult agricultural pest *Tetranychus urticae* were observed while some effects on the protonymphs were seen at concentrations similar to the current application. Based on these results it can be concluded that the active substance and the product have no risk to non-target arthropods.

B.9.5 Effects on earthworms

B.9.5.1 Toxicity to earthworms

Plant protection product

Reference:	Benz, G. and Altweg, A (1975) Safety of Bacillus thuringiensis for earthworms Published, Journal of Invertebrate Pathology 26, 125-126 (1975)
Guideline:	N/A
GLP:	No
Material and methods:	Test material: Dipel (B. thuringiensis, serotype H3; potency - 16,000 IUP/mg); Bactospeine (B. thuringiensis, serotype H1; potency - 1000 IUP/mg).Recommended dose for application in the field = 60 mg/m^2 containing 1.5 x 10^9 spores. Dipel was applied at three rates, 60, 600 and 6000 mg/m^2 (corresponding to 1.5 x 10^{13}, 1.5 x 10^{14} and 1.5 x 10^{15} CFU/ha) , to field plots of 9 m^2 (3 x 3 m) in an ash and maple forest. The two lower rates were suspended in 5 L of

	water and the highest rate in 10 L, with application being carried out using a backpack sprayer. Bactospeine was applied in 5 L of water at a rate of 30 mg/m^2 and there was also a water-treated control (10 L water). The soil in the plots was rich in humus and clay and before treatment the leaf litter was carefully removed and then replaced immediately after application. A pre-treatment assessment of the earthworm populations was made on the plots, by removing the soil from a 50 cm square to a depth of 30 cm and counting all the earthworms found (which were then replaced in the hole). Similar assessments were made 3, 6 and 9 weeks after application, with the earth-worms found being examined for signs of disease.
Test substance:	Dipel (Bacillus thuringiensis serotype H3, 16,000 IU/mg); Bactospeine
Test species:	Earthworm (species not specified)
Number of test animals:	About 10 worms x 3-4 replicates
Treatments:	Dipel: 60, 600 and 6000 mg/mq; Bactospeine 30 g/mq
Duration:	3,6 and 9 weeks
Test conditions:	Field
Deviations from guide-line	-
Endpoint:	The pre-treatment mean earthworm density in the soil from the five untreated plots was 29.8/75 litres of soil. There were no statistically significant differ-ences in worm density between the treated and control treatments in the assessments carried out after application. Only a few significant differences between individual treatments were found and these showed no clear asso-ciation with the application received. There were no marked reductions in earthworm numbers over time and the mean post-treatment numbers actual-ly showed a slight increase in worm density on all plots. The results are summarised in Table 1.

Table 1

Mean number of earthworms/75 L soil in Bacillus thuringiensis treated field plots

	Control (water only)	Dipel (60 mg/m^2)	Dipel (600 mg/m^2)	Dipel (6000 mg/m^2)	Bactospeine (30 mg/m^2)
Pre-treatment	30	27	31	34	27
+3 weeks	31	40 a	39	46 bcde	29 e
+6 weeks	32	28 c	36	26 b	35
+9 weeks	32	24 ad	38	38	33
Mean (post-treatment)	31.2	29.7	36.0	36.0	31.0

* Mean values followed by different letter, significantly different (p = 0.05)

Observations:	On the basis of these results it was concluded that B. thuringiensis did not have an adverse effect on earthworms populations when applied at rates in excess of those used in the field (up to 6000 mg Dipel/m^2 and 30 g Bactospeine/m^2).

Results:

A summary of endpoints is given in the table below.

Table B.9.5.1: Toxic effects / Infectivity / Pathogenicity of plant protection product to earthworms

Test species	No specific species test, field study on suborder level 'earthworms'
Toxicity of plant protection product	No effects on earthworm populations discovered within 9 weeks after treatment at dose levels of up to 6000 mg product/m2, which equals 1.5 x 10^{15} CFU/ha.
Pathogenicity/ Infectivity	No signs of infectivity or pathogencity observed.

Comments and conclusion RMS:

This scientific paper was previously evaluated in the DAR of 2007. Shortcomings were notes as follows: *'This experiment shows some limits: the earthworm species is not defined, the subspecies of Bt used is not aizawai; the statistical analysis of data is not clearly shown in the table; description of experiment is generical; experiment was not carried out under GLP conditions.'*

Some additions were made to the study summary by the RMS:

Added: Recommended dose for application in the field = 60 mg/m^2 containing 1.5 x 10^9 spores.
Dipel was applied at three rates, 60, 600 and 6000 mg/m^2 *added:*(corresponding to 1.5 x 10^{13}, 1.5 x 10^{14} and 1.5 x 10^{15} CFU/ha).

The applicant did not submit this study, but the study is included here, because it was part of the previous evaluation DAR 2007). The RMS maintains the issues and conclusions identified in the DAR of 2007 and considers this field study only suitable as supporting information.

B.9.5.2 Infectiveness to earthworms

The 30 days study with the active substance did not identify any adverse effects on earthworms.

B.9.5.3 Pathogenicity to earthworms

The 30 days study with the active substance did not identify any adverse effects on earthworms.

B.9.5.4 Summary and risk assessment for earthworms

No effects on earthworms of the active substance at 1000 mg a.s./kg dry soil were observed in a 30 day study. This concentration is above the PECsoil of 57.6 mg a.s./kg soil assuming no considerations for degradation. The risk to earthworms is therefore considered acceptable.

B.9.6 Effects on non-target soil micro-organisms

No studies are available to assess the effects of XenTari® WG or BTa ABTS-1857 on soil micro-organisms. However, there is published information that assesses the impact of various other *B. thuringiensis* subspecies. Given the nature of the host specificity of the different subspecies (between different insect groups) and the lack of taxonomic relatedness between these susceptible species and soil micro-organisms, this information is considered relevant here.

EFSA (2013) concluded that *"Bacillus thuringiensis* occurs naturally and ubiquitously in the environment. It is a common component of the soil micro-flora and has been isolated from most terrestrial habitats. Although originally recovered mainly from insects, recent studies have indicated that *B. thuringiensis* is distributed in soil sparsely but frequently and its distribution is widespread, both locally and worldwide. *B. thuringiensis* is not adapted to survive as an active member of the soil microbial community and the low potential for spore germination, growth and resporulation restricts population growth. Although specific studies were not produced by the Applicant, it can be suggested that there will be low impact on soil micro-organism populations and processes when XenTari® WG is applied according to the proposed GAP, and intended uses".

Following field applications of formulations containing *B. thuringiensis* endospores and parasporal crystals, the vegetative cells and crystal proteins of *B. thuringiensis* are rapidly degraded in soil. The actions of indigenous micro-organisms, which compete for nutrients and produce proteolytic enzymes that degrade the protoxin, lead to a rapid loss of potency and insecticidal activity in soil (e.g. with a half-life of 3-6 days). In a study to investigate the effects of pH, moisture, nutrient availability and indigenous micro-organisms on the survival and growth of *Bacillus thuringiensis* and *B. cereus* spores in a fallow, sandy silt loam soil, nutrient availability was shown to be the factor of greatest importance (DAR, B.8). The study demonstrated that *B. thuringiensis* subsp. *aizawai* could not grow under most natural soil conditions due to lack of competitive ability with other soil micro-organisms. This suggests that *B. thuringiensis* subsp. *aizawai* is not adapted to survive as an active member of the soil microbial community. In a further study, the population dynamics of vegetative cells and spores of *B. thuringiensis* subsp. *thuringiensis* and subsp. *morrisoni* were studied in three Japanese agricultural soils (Anon, 2005; DAR, B.8). The results showed that *B. thuringiensis* spores were capable of germinating in autoclaved soil samples but not in natural, non-sterilised soils and that vegetative cells inoculated into natural soil were able to form spores but the cells disappeared rapidly within 1-2 days. These results support the earlier findings, and suggest that the spore is the only state in which *B. thuringiensis* can persist in natural soils. The persistence of *Bacillus thuringiensis* spores as inert particles in the soil will have little or no effect on the native micro-flora.

The photodegradation effects of sunlight also affect the survival and growth of endospores and vegetative cells in the environment. Viable spores are expected to survive but remain inactive and immobile in soil for several months or even years, during which time a natural breakdown occurs, resulting in gradual spore mortality. The endospores rarely germinate in soil unless favourable conditions exist in combination such as neutral to alkaline pH, sufficient nutrient availability, favourable soil temperature and moisture content and lack of competition/predation from other soil micro-organisms. It is thus concluded that *B. thuringiensis* is not adapted to survive as an active member of the soil microbial community and the low potential for spore germination, growth and re-sporulation restricts population growth.

This assessment is consistent with the specific information presented for the effects of *B. thuringiensis* on micro-organisms (although not conducted with *B. thuringiensis* subsp. *aizawai*, the nature of its host specificity suggests that this information is relevant). Thus, the toxins from *B. thuringiensis* subsp. *kurstaki*, *tenebrionis* and *israelensis* did not affect the growth of a variety of bacteria (8 Gram-negative, 5 Gram-positive and a cyanobacterium), fungi (2 Zygomycetes, 1 Actinomycete, 2 Deuteromycetes, and 2 yeasts), and algae (primarily green and diatoms) in pure and mixed culture. Similarly, studies on *B. thuringiensis* subsp. *kurstaki* have shown that at field application rates there is no evidence of any effects on the soil microflora as measured by C and N mineralisation dynamics (basal soil respiration, substrate induced respiration and microbial biomass, microbial metabolic quotients NH_4-N and NO_3-N levels, and cellulose decay potential).

It can therefore be concluded that there will be no significant impact on soil micro-organism populations and processes when XenTari® WG is applied according to the proposed GAP.

Comment by RMS: The applicant is requested to add the references in the text provided above.

B.9.7 Effects on terrestrial plants

No studies submitted. Due to the mode of action, not expected to cause any effects on terrestrial plants.

B.9.8 Additional studies

Reference:	Barnes, S.P. (2004) *Bacillus thuringiensis* subspecies *israelensis*, strain SA3A. Activated sludge- respiration inhibition test. Unpublished Report No. ZAB 040/033471
Guideline:	OECD 209; EC 88/302
GLP:	Yes
Material and methods:	The effect of BTi (*Bacillus thuringiensis* subspecies *israelensis,* strain SA3A, LOT 105-383-Q8, trade name Teknar technical concentrate VBC-60056, purity 100%) on the respiration rate of activated sludge was assessed by the methods detailed in OECD 209. Samples of activated sludge (suspended solids 1.6 g/L), fed with synthetic sewage, were exposed to the test substance at nominal concentrations of 1, 10 and 100 mg/L for three hours. The rates of oxygen consumption were determined and compared with those of controls containing activated sludge and synthetic sewage alone, which were established at the beginning and end of the culture series.
Test substance:	BTi (*Bacillus thuringiensis* subspecies *israelensis,* strain SA3A)
Test species:	Respiration of activated sludge
Number of test animals:	NA
Treatments:	Nominal concentrations of 1, 10 and 100 mg/L, controls (synthetic sludge and activated sludge)
Duration:	3h exposure period, 10 min measurement
Test conditions:	pH of the activated sludge was 7.4 pH in treatments increased from 7.6 -8.0 in the treatments and the control Temperature increased from 19.4 to 20.3 in the treatments, control 19.9-19.8
Deviations from guideline	
Endpoint:	rates of oxygen consumption
Observations:	BTi had no inhibitory effect on the respiration rate of activated sludge at any of the concentrations tested, but sludge respiration rates were increased by be-

	tween 15 and 21% of the mean control rate in the three mixtures containing BTi at 100 mg/L. They were also increased by 11% at 10 mg/L and by 2% at 1 mg/L, but it was not possible to conclude that these effects were dose-related owing to the sensitivity and low replication employed for this test system. The 3-hour EC_{20}, EC_{50} and EC_{80} values were not determined as a result of this study.

Results:

A summary of endpoints is given in the table below.

Table B.9.5.1: Effects on sewage treatment plants (activated sludge)

Test species	Activated sludge (test carried out with Bacillus thuringiensis israelensis strain SA3A)
Toxicity of plant protection product	No significant effects seen on respiration inhibition at the concentrations tested (1, 10 and 100 mg technical concentrate/L)
Pathogenicity/ Infectivity	Not tested.

Comments and conclusion RMS:

Submitted by the applicant for the purpose of renewal.

The study was conducted according to OECD 209, which is considered acceptable.
A positive control treatment was included, which proved to be sensitive to respiratory inhibition. The initial oxygen concentrations were in the normal range and in the treatments comparable to the control treatments. Therefore the test is considered valid.
Bti had no inhibitory effect at any of the concentrations tested (1,10, 100 mg test item/L). At 100 mg /L respiration was increased by 15 and 21%, at 10mg/L by 11% and at 1 mg/L by 2%. These results however remain inconclusive due to a lack of appropriate statistical replication and the sensitivity of the measurement parameters (oxygen consumption).
The study was however not carried out with the subspecies applied for here (BTa), but instead with Bt *israeliensis* (Bti). Therefore this information cannot be used in the risk assessment for Bta.

B.9.9 References relied on

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
IIIM 10.2/01	Dorgerloh, M.	2001	XenTari WG - Influence on the growth of the green alga, Selenastrum capricornutum Report No. E 323 2084-2 Source: Bayer AG GLP Unpublished **PREVIOUSLY EVALUATED. IN DAR (2008)**	N	N	N/A	VBC
IIIM 10.3/01	Rhodes, J.E., Downing, J.	1997	Toxicological effect of XenTari WDG (ABG-6314) on a colony of honey bees Report No. 43052 Source: ABC Laboratories, Inc. GLP Unpublished **PREVIOUSLY EVALUATED. IN DAR (2008)**	N	N	N/A	VBC
IIIM 10.3/02	Bolckmans, K.	1995	Toxicity of the biological insecticide ABG-6314 for bumblebees (Bombus terrestris L.) Report No. 9505151F Source: BIOBEST N.V. Non-GLP Unpublished **PREVIOUSLY EVALUATED. IN DAR (2008)**	N	N	N/A	VBC

IIIM 10.4/01	Schuld, M.	2002	XenTari WG: Toxicity test on the egg parasitoid, Trichogramma cacoeciae Marchal (Hymenoptera, Trichogrammatidae) under laboratory conditions Report No. 20011423/01-NLTc Source: GAB Biotechnologie & IFU Umweltanalytik GLP Unpublished **PREVIOUSLY EVALUATED. IN DAR (2008)**	N	N	N/A	VBC
IIIM 10.5/01	Benz, G., Altweg, A.	1975	Safety of Bacillus thuringiensis for earthworms Journal of Insect Pathology 26, 125-126 Not GLP; Published **PREVIOUSLY EVALUATED. IN DAR (2008)**		N		--
IIIM 10.7/01	Barnes, S.P.	2004	*Bacillus thuringiensis* subspecies *israelensis*, strain SA3A. Activated sludge- respiration inhibition test. Report No. ZAB 040/033471 Source: Huntingdon Life Sciences Ltd., Cambridgeshire, UK GLP Unpublished **SUBMITTED FOR THE PURPOSE OF RENEWAL**	N	Y	New data submitted for first time	VBC