Renewal Assessment Report

Bacillus thuringiensis ssp. aizawai strain ABTS-1857

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 marked grey.

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.

Bacillus thuringiensis isolates also synthesize insecticidal proteins during the vegetative growth phase, which are subsequently secreted into the growth medium. These proteins are commonly known as vegetative insecticidal proteins (VIPs) and hold insecticidal activity against lepidopteran, coleopteran and some homopteran pests.

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

No new studies are submitted assessing the effect of BTa ABTS-1857 on birds. The risk assessment is based on data that have already been evaluated as part of the EU review for the inclusion of this

strain in Annex I. Experiments on toxicity and pathogenicity of the MPCA were performed on bobwhite quail (*Colinus virginianus*) and mallard duck (*Anas platyrhynchos*). The summaries of the studies as per original DAR and the current evaluation by RMS are included below.

B.9.1.1 Toxicity to birds

Microbial pest control agent (MPCA)

Reference 8.1/01

Reference:	[redacted] (1991a) ABG-6305: An avian oral pathogenicity and toxicity study in the bobwhite. [redacted], Unpublished report No.: 161-117
Guideline:	US EPA Pesticide Assessment Guidelines. FIFRA. Hazard Evaluation: Wildlife and Aquatic organisms. Subdivision M, Microbial Pest Control Agents, Subsection 154A-16. Office of Pesticides Programs, November 1988
GLP:	Yes (40 CFR Part 160). Except samples of the dosing solutions were not collected for confirmation of the test concentrations, homogeneity or stability
Material and methods:	Test material: ABG-6305 Technical Powder. Lot No 42-221-BD. *Bacillus thuringiensis* subsp. *aizawai*, strain ABTS-1857, fermentation solids and solubles – 100% (2 x 10^{11} cfu/g). Eight groups of five bobwhite quail (*Colinus virginianus*), not differentiated by sex, were acclimated from the day of receipt until test initiation when the birds were 21 days old. Six groups were treated with ABG-6305 administered as a suspension in distilled water by oral gavage at 1.0% of body weight (w/v). Each bird received 1714 mg test substance/kg b.w. (approximately 3.4 x 10^{11} cfu/kg b.w.), daily for five days. Two groups of five birds each served as negative controls and were administered distilled water at 1.0% of body weight (w/v) by oral gavage, daily for five days. Each dose group was assigned to a wire-mesh pen (approximate floor space 72 cm x 90 x 23 cm high). Food and water were provided ad libitum throughout the acclimation and testing periods. Birds were maintained at an average ambient room temperature of 21.8 ± 1°C, with an average relative humidity of 43% ± 11% and a photoperiod of 16 hours light: 8 hours dark. Following test initiation (Day 0) until termination on Day 30, all birds were observed at least twice daily. A record was maintained of all mortality, signs of toxicity, and abnormal behaviour. Individual body weights were recorded on Days 0, 1, 2, 3 and 4 prior to dosing and on Days 11, 18, 25 and 30. Average estimated feed consumption was measured for Days 0-4, 4-11, 11-

	18, 18-25 and 25-30.
Micro-organism:	ABG-6305 Technical Powder. Lot No 42-221-BD. *Bacillus thuringiensis* subsp. *aizawai*, strain ABTS-1857
Test species:	bobwhite quail (*Colinus virginianus*): 30 treated and 10 control
Number of test animals:	Eight groups of five bobwhite quail
Treatments:	Six groups were treated with ABG-6305 administered as a suspension in distilled water by oral gavage at 1.0% of body weight (w/v). Each bird received 1714 mg test substance/kg b.w. (approximately 3.4 x 10^{11} cfu/kg b.w.), daily for five days. Two groups of five birds each served as negative controls and were administered distilled water at 1.0% of body weight (w/v) by oral gavage, daily for five days.
Duration:	5 days of treatment and 30 of observation
Test conditions:	Each dose group was assigned to a wire-mesh pen (approximate floor space 72 cm x 90 x 23 cm high). Food and water were provided ad libitum throughout the acclimation and testing periods. Birds were maintained at an average ambient room temperature of 21.8 ± 1°C, with an average relative humidity of 43% ± 11% and a photoperiod of 16 hours light: 8 hours dark.
Deviations from guideline	None
Endpoint:	The results are presented in Table 1. No mortalities occurred in any of the treatment groups and no overt signs of treatment-related toxicity or pathogenicity were observed during the study. When compared to the negative control groups, there were no apparent treatment-related effects upon body weight gain in any of the treatment groups. Feed consumption was variable among pens (due to excessive wastage by some birds) but when compared to the negative control groups, there did not appear to be any effect in the treatment groups. At test termination, all birds were euthanized and subjected to gross necropsy. No treatment-related lesions were observed in any birds from the control or treatment groups.

Table 1

Mortality, feed consumption and body weight changes in bobwhite following dosing with ABG-6305

Dosage (mg test substance/kg)	% mortality (Day 30)	Change in group mean body weight (g), days 0 - 30	Group mean feed consumption (g/bird/day)*		
			0 - 4	11 - 18	25 – 30
Control (water only)	0	85	14	20	23
Control (water only)	0	86	15	28	28
1714	0	94	23	24	31
1714	0	89	19	24	20
1714	0	93	19	24	26
1714	0	91	11	20	24
1714	0	91	23	24	28
1714	0	98	19	21	22

* Representative periods (Days 4-11 and 18-25 omitted).

Observations:	ABG-6305 Technical Powder showed no apparent pathogenicity, toxicity or effect upon survival of young bobwhite quail when administered by oral gavage at 1714 mg a.s./kg b.w. (approximately 3.4 x 10^{11} cfu/kg b.w/d.) for five days.

Results:

A summary of endpoints is given in the table below.

Table B.9.1.1.a: Toxicity effects/ Infectivity / Pathogenicity of the MPCA to bird

Test species	Bobwhite quail *(Colinus virginianus)*
Toxicity	LD50: > 1714 mg test substance/kg b.w. >3.4 x 10^{11} cfu/kg b.w.
Infectivity / Pathogenicity	No signs of infectivity and pathogenicity observed

Comments and conclusion RMS:

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

The most recent guideline applicable to this test is OPPTS 885.4050 (1996). As the test was performed in 1990, the predecessor of this guideline was used. This is considered acceptable; however a comparison to the requirements in the current guidelines (OPPTS 885.4050) was made.

No statistical analysis was performed to detect differences in feed consumption and body weight gain. A slightly higher weight gain as compared to the controls was observed for the treatments, but no weight decrease was found.

The number of birds tested per treatment group was 5, whereas as per OPPTS guideline at least 10 birds are required. However, considering that the study was conducted in 1991 and as 6 dose levels were tested in the interest of animal welfare this considered acceptable.

Animal housing in groups instead of individually and a prolonged photoperiod of 16h did not seem to have an influence on the outcome of the test.

No signs of treatment related pathogenicity or toxicity was observed. Infectivity was not investigated.

The study is considered relevant and reliable. The endpoints can be used in risk assessment.

Reference 8.1/02

Reference:	[redacted] (1991b) ABG-6305: An avian oral pathogenicity and toxicity study in the mallard. [redacted], Unpublished report No.: 161-118
Guideline:	US EPA Pesticide Assessment Guidelines. FIFRA. Hazard Evaluation: Wildlife and Aquatic organisms. Subdivision M, Microbial Pest Control Agents, Subsection 154A-16. Office of Pesticides Programs, November 1988
GLP:	Yes (40 CFR Part 160). Except samples of the dosing solutions were not collected for confirmation of the test concentrations, homogeneity or stability
Material and methods:	Test material: ABG-6305 Technical Powder. Lot No 42-221-BD. Bacillus thuringiensis subsp. aizawai, strain ABTS-1857, fermentation solids and solubles – 100% (2 x 10^{11} cfu/g). Eight groups of five mallard ducks (*Anas platyrhynchos*), not differentiated by sex, were acclimated from the day of receipt until test initiation when the birds were 21 days old. Six groups were treated with ABG-6305 administered as a suspension in distilled water by oral gavage at 1.0% of body weight (w/v). Each bird received 1714 mg test substance/kg b.w. (approximately 3.4 x 10^{11} cfu/kg b.w.), daily for five days. Two groups of five birds

	each served as negative controls and were administered distilled water at 1.0% of body weight (w/v) by oral gavage, daily for five days. Each dose group was assigned to a wire-mesh pen (approximate floor space 75 cm x 90 x 45 cm high). Food and water were provided ad libitum throughout the acclimation and testing periods. Birds were maintained at an average ambient room temperature of 21.8 ± 1°C, with an average relative humidity of 43% ± 11% and a photoperiod of 16 hours light: 8 hours dark. Following test initiation (Day 0) until termination on Day 30, all birds were observed at least twice daily. A record was maintained of all mortality, signs of toxicity, and abnormal behaviour. Individual body weights were recorded on Days 0, 1, 2, 3 and 4 prior to dosing and on Days 11, 18, 25 and 30. Average estimated feed consumption was measured for Days 0-4, 4-11, 11-18, 18-25 and 25-30.
Test substance:	ABG-6305 Technical Powder. Lot No 42-221-BD. Bacillus thuringiensis subsp. aizawai, strain ABTS-1857
Test species:	mallard ducks (*Anas platyrhynchos*)
Number of test animals:	Eight groups of five mallard ducks (*Anas platyrhynchos*): 30 treated and 10 control
Treatments:	Six groups were treated with ABG-6305 administered as a suspension in distilled water by oral gavage at 1.0% of body weight (w/v). Each bird received 1714 mg test substance/kg b.w. (approximately 3.4×10^{11} cfu/kg b.w.), daily for five days. Two groups of five birds each served as negative controls and were administered distilled water at 1.0% of body weight (w/v) by oral gavage, daily for five days.
Duration:	5 days of treatment and 30 of observation
Test conditions:	Each dose group was assigned to a wire-mesh pen (approximate floor space 75 cm x 90 x 45 cm high). Food and water were provided ad libitum throughout the acclimation and testing periods. Birds were maintained at an average ambient room temperature of 21.8 ± 1°C, with an average relative humidity of 43% ± 11% and a photoperiod of 16 hours light: 8 hours dark.
Deviations from guideline	None
Endpoint:	The results are presented in Table 2. No mortalities occurred in any of the treatment groups and no overt signs of treatment-related toxicity or pathogenicity were observed during the study. When compared to the negative control groups, there were no apparent treatment-related effects upon body weight gain in any of the treatment groups. Feed consumption was variable

	among pens (due to excessive wastage by some birds) but when compared to the negative control groups, there did not appear to be any effect in the treatment groups. At test termination, all birds were euthanized and subjected to gross necropsy. No treatment-related lesions were observed in any birds from the control or treatment groups.

Table2

Mortality, feed consumption and body weight changes in mallard following dosing with ABG-6305

Dosage (mg test substance/kg)	% mortality (Day 30)	Change in group mean body weight (g), days 0 - 30	Group mean feed consumption (g/bird/day)*		
			0 - 4	11 - 18	25 – 30
Control (water only)	0	468	83	139	141
Control (water only)	0	437	86	148	159
1714	0	615	107	157	165
1714	0	597	114	157	165
1714	0	693	111	198	167
1714	0	718	97	210	209
1714	0	495	99	159	145
1714	0	441	98	183	149

***** Representative periods (Days 4-11 and 18-25 omitted).

Observations:	ABG-6305 Technical Powder showed no apparent pathogenicity, toxicity or effect upon survival of young mallard ducks when administered by oral gavage at 1714 mg test substance/kg b.w. (approximately 3.4 x 10^{11} cfu/kg b.w.) for five days.

Results:

A summary of endpoints is given in the table below.

Table B.9.1.1.a: Toxicity effects/ Infectivity / Pathogenicity of the MPCA to bird

Test species	Mallard duck *(Anas platyrhynchos)*

Toxicity	LD50: > 1714 mg test substance/kg b.w. > 3.4 x 10^{11} cfu/kg b.w.
Infectivity / Pathogenicity	No signs of infectivity and pathogenicity observed

Comments and conclusion RMS:

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

The most recent guideline applicable to this test is OPPTS 885.4050 (1996). As the test was performed in 1990, the predecessor of this guideline was used. This is considered acceptable, however a comparison to the requirements in the current guidelines (OPPTS 885.4050) was made.

No statistical analysis was performed to detect differences in feed consumption and body weight gain. A slightly higher weight gain as compared to the controls was observed for the treatments, but no weight decrease was found.

The number of birds tested per treatment group was 5, whereas as per OPPTS guideline at least 10 birds are required. However, considering that the study was conducted in 1991 and as 6 dose levels were tested in the interest of animal welfare this considered acceptable.

Animal housing in groups instead of individually and a prolonged photoperiod of 16h did not seem to have an influence on the outcome of the test.

No signs of treatment related pathogenicity or toxicity were observed. Infectivity was not investigated.

The study is considered relevant and reliable. The endpoints can be used in risk assessment.

Toxin/metabolite from microbial pest control agent (MPCA)

No study or information was submitted. According to the information provided in the former DAR: "*Bacillus thuringiensis* subsp. *aizawai,* Strain ABTS-1857, like other Bt strains commercially available, has been shown not to contain β-exotoxins or enterotoxins. Strict maintenance of environmental conditions and quality control analysis during the manufacturing process ensures the absence of potential microbial and non-microbial contaminants or potential animal or human pathogens".

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. Therefore, the pathogenicity of Bt to insects involves targeting specific cadherin receptors in the host; indicating that the mode of action involves effects on host-specific cell adhesion proteins.

Considering the mode of action, it is not expected that these host-specific toxins will be active in the birds gastrointestinal tract as they will be inactivated by the protease enzymes (please refer also to the Vol. 3 B6).

Infectiveness to birds

No data submitted by the applicant.

B.9.1.2 Pathogenicity to birds

Summary of the studies on birds on toxicity and pathogenicity.

Table 9.1.3.a: Summary of the studies on effects on birds treated with MPCA

Species	Test duration	Dose range	Results/ Endpoint	Observations	Reference
TOXICITY					
Bobwhite quail *Colinus virginianus*	30 days	1714 mg test substance/kg b.w. (3.4×10^{11} cfu/kg b.w.)	LD50: > 1714 mg test substance/kg b.w. (> 3.4×10^{11} cfu/kg b.w.)	No signs of pathogenicity were found.	[redacted] *1991a*
Mallard duck *Anas platyrhynchos*	21 days	1714 mg test substance/kg b.w. (3.4×10^{11} cfu/kg b.w.)	LD50: > 1714 mg test substance/kg b.w. (> 3.4×10^{11} cfu/kg b.w.)	No signs of pathogenicity were found.	[redacted] *1991b*
INFECTIVENESS					
No studies or information submitted.					
PATHO-GENICITY					
Bobwhite quail *Colinus virginianus*	30 days	1714 mg test substance/kg b.w. (3.4×10^{11} cfu/kg b.w.)	LD50: > 1714 mg test substance/kg b.w. (> 3.4×10^{11}	No signs of pathogenicity were found. Necropsy did not show any	[redacted] *1991a*

Species	Test duration	Dose range	Results/ Endpoint	Observations	Reference
			cfu/kg b.w.)	abnormalities.	
Mallard duck *Anas platyrhynchos*	21 days	1714 mg test substance/kg b.w. (3.4×10^{11} cfu/kg b.w.)	LD50: > 1714 mg test substance/kg b.w. (> 3.4×10^{11} cfu/kg b.w.)	No signs of pathogenicity were found. Necropsy did not show any abnormalities.	[redacted] *1991b*

Table 9.1.3.b: Summary of the studies on effects on birds treated with toxin/metabolite

Species	Test duration	Dose range	Results/ Endpoint	Observations	Reference
TOXICITY					
No data required for this aspect.					

B.9.2 Effects on aquatic organisms

No new studies are submitted assessing the effect of BTa ABTS-1857 on aquatic organisms. The studies' summaries of the studies as per original DAR and the current evaluation by RMS are included below.

B.9.2.1 Effects on fish

B.9.2.2 Toxicity to fish

Microbial pest control agent (MPCA)

Reference 8.2.1/01

Reference:	[redacted] (1991a) Acute Toxicity of ABG-6305 to the Rainbow Trout Oncorhynchus mykiss. [redacted] Unpublished report No.: 9107-A
Guideline:	Based on US EPA-540/9-85-006 (1985) and US EPA Pesticide Assessment Guidelines. Subdivision E, Hazard Evaluation: Wildlife and Aquatic organisms. Ecological Effects Branch, Hazard Evaluation Division, Office of Pesticides Programs, March 1988
GLP:	Yes (40 CFR Part 160).

Material and methods:	Test material: ABG-6305. Lot No 42-221-BD. Bacillus thuringiensis subsp. aizawai, strain ABTS-1857 (potency 28151 IU/mg, 2 x 10^{11} cfu/g). A semi-static test system with daily renewal was used to determine the 24, 48, 72 and 96-hour LC_{50} values for rainbow trout (Oncorhynchus mykiss) exposed to ABG-6305 in freshwater. The test vessels (glass aquaria with a capacity of 19.6 litres) were filled with 15 L volume of test media. Appropriate amounts of the test substance were added directly to each test vessel and thoroughly mixed. Nominal concentrations of the test substance were: 0 mg/L (control) and 100 mg/L ABG-6305 (equivalent to 2 x10^7 spores / ml). The media in each vessel was renewed daily during the 96 hour test. Thirty rainbow trout were randomly and equally distributed among three replicates for each treatment. The weight and length of the water only control fish at the end of the exposure period were 0.55 g and 4.1 cm respectively. The loading density during the toxicity test was 0.37 g fish/L. The fish were not fed during the exposure period. The test was conducted at a target temperature of 12 ± 2 °C. Aeration was not required to maintain dissolved oxygen concentrations above acceptable levels. The photoperiod used was 16 hours light and 8 hours dark. The number of surviving organisms and the occurrence of sublethal effects (loss of equilibrium, erratic swimming, loss of reflex, excitability, discoloration or change in behaviour) were determined visually and recorded. Dissolved oxygen, pH, conductivity and temperature were measured and recorded daily. The sponsor undertook measurement of test substance concentration. Samples were collected from each test vessel at the initiation and termination of the test and before and after media renewal at 24, 48, and 72 hours.
Micro-organism	ABG-6305. Lot No 42-221-BD. Bacillus thuringiensis subsp. aizawai, strain ABTS-1857 (potency 28151 IU/mg, 2 x 10^{11} cfu/g).
Test species:	Rainbow trout (Oncorhynchus mykiss)
Number of test animals:	Thirty rainbow trout were randomly and equally distributed among three replicates for each treatment.
Treatments:	A semi-static test system with daily renewal was used to determine the 24, 48, 72 and 96-hour LC_{50} values for rainbow trout (Oncorhynchus mykiss) exposed to ABG-6305 in freshwater
Duration:	96 hours
Test conditions:	The test was conducted at a target temperature of 12 ± 2 °C. Aeration was not required to maintain dissolved oxygen concentrations above acceptable

	levels. The photoperiod used was 16 hours light and 8 hours dark.
Deviations from guide-line	The bacteriological analysis of test media was conducted by the sponsor.
Endpoint:	All test vessels containing ABG-6305 were cloudy with undissolved material on the bottom. Bacteriological analysis of water samples gave a mean measured spore concentration of 6.3 x 10^6 spores / ml in new suspensions and 6.55 x 10^6 spores / ml for old suspensions prior to renewal (average for 3 replicates) in the treatment vessel. The spore count from the water analysis generally confirm the nominal concentration of 2 x10^7 spores / ml. No mortality or sublethal effects were observed in any test vessel during the 96-hour exposure. ABG-6305 was not toxic to rainbow trout at the tested concentration. The 24, 48, 72 and 96-hour LC_{50} values are greater than 100 mg/L.
Observations:	Exposure of the test organism to the test material resulted in a 96-hour LC_{50} greater than 100 mg/L ABG-6305, the highest tested concentration (equivalent to a nominal concentration of 2 x10^7 spores / ml). The 96-hour NOEC is 100 mg/L ABG-6305. No sublethal effects were observed in any test vessel during the 96-hour exposure.

Results:

A summary of endpoints is given in the table below.

Table B.9.2.2.a: Toxicity effects/ Infectivity / Pathogenicity of the MPCA to fish

Test species	Rainbow trout (*Onchorhyncus mykiss*)
Toxicity	96 hour LC_{50}: $>$ 2.0 x 10^7 CFU/L (nominal).
Infectivity / Pathogenicity	No signs of pathogenicity. Infectivity was not investigated. The study duration was too short for the investigation of pathogenicity.

Comments and conclusion RMS:

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

The most recent guideline applicable to this test is OPPTS 885.4200 (1996). As the test was performed in 1991, the predecessor of this guideline was used. Principally, this is considered acceptable, however a comparison to the current guideline was made.

The initial mean weight and range of weight of the test animals is not reported, however, the length criterion is fulfilled and therefore it can reasonably be assumed that the weight criterion would not have changed the outcome of the test.

No signs of treatment related toxicity were observed. The RMS considers the study duration too short for the observation of any pathogenic effects. Infectivity was not studied.

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

Reference 8.2.1/02

Reference:	[redacted] (1993) Xentari Technical Powder (ABG-6305) - Infectivity and Pathogenicity to Rainbow Trout (*Oncorhynchus mykiss*) during a 20-day static renewal test. [redacted]., Unpublished report No.: 93-6-4837
Guideline:	FIFRA Guideline 154A-19. Static renewal protocol in compliance with US EPA Guidelines for testing microbial pest control agents (US EPA 1989: 154A-19)
GLP:	Yes (40 CFR Part 160) with the following exceptions: routine water and food contamination screening analysis for pesticides, PCB's and metals and turbidity analysis were conducted using standard US EPA procedures by Lancaster Laboratories, Lancaster, PA
Material and methods:	Test material: ABG-6305. Lot No 42-221-BD. *Bacillus thuringiensis* subsp. *aizawai*, strain ABTS-1857 (potency 28151 IU/mg, 2 x 10^{11} cfu/g). A semi-static test system with renewal at 3-4 day intervals was used to determine the potential for infectivity and pathogenicity of rainbow trout following prolonged, continuous exposure to ABG-6305 at aqueous and dietary concentrations equivalent to 500 and 1000 times expected environmental concentrations (EEC), respectively. The test vessels (glass aquaria with a capacity of 18.9 litres) were filled with 15 L volume of test media. For the aqueous administration a stock suspension was prepared by mixing 24.5 g of ABG-6305 with 1000 ml of dilution water. This initial stock solution was then diluted to 50 L with dilution water giving a nominal concentration of 7.36 x 10^{10} cfu/L and after mixing, 15 L was transferred to each replicate test vessel. Three control aquaria were also maintained during this study. All replicate treatment level and control solutions were renewed on exposure days 4, 7, 11, 14 and 18. Sixty rainbow trout were impartially selected and equally distributed among the test and control aquaria: 10 organisms per replicate vessel, 30 organ-

	isms per treatment and control. The weight and length of the water only control fish at the end of the exposure period were 0.52 g and 4.0 cm respectively. The loading density during the toxicity test was 0.35 g fish/L. The fish were fed once daily during the exposure period with a commercially prepared finely ground pellet food, mixed with the test material at a nominal concentration of 1000 times the EEC. Based on preliminary studies a nominal dietary concentration of 1.47 x 10^8 cfu/g was selected for the definitive study. The test was conducted at a target temperature of 12 ± 2 °C. Aeration was maintained constantly throughout the exposure period to maintain both a homogeneous mixture of the test material and acceptable oxygen concentrations. The photoperiod used was 16 hours light and 8 hours dark. Dissolved oxygen, pH and temperature were measured and recorded daily. Observations of mortality, abnormal behaviour and gross pathogenic response were made once daily. At test termination (day 20) all surviving fish were examined for signs of adverse effects, measured and weighed. Following external examination, thirty randomly selected fish exposed to the control solution and all the surviving fish exposed to the treatment levels were preserved and sent for complete histopathological examination. Additionally all test organisms that had died and were preserved during the 20-day study were sent for examination. Measurement of colony forming units /L in the test solutions and control solution was made from each test vessel at initiation and at each media renewal (newly prepared exposure solution only). For the duration of the definitive test, concentration verification of each batch of the amended food mix and each batch of the control food was routinely conducted. A food dissociation study was conducted prior to the initiation of the definitive study. This indicated that >99% of the test material remained associated with the food following 60 minutes of exposure in dilution water.
Test substance	Test material: ABG-6305. Lot No 42-221-BD. *Bacillus thuringiensis* subsp. *aizawai*, strain ABTS-1857 (potency 28151 IU/mg, 2 x 10^{11} cfu/g).
Test species:	Rainbow Trout (*Oncorhynchus mykiss*)
Number of test animals:	Sixty rainbow trout were impartially selected and equally distributed among the test and control aquaria: 10 organisms per replicate vessel, 30 organisms per treatment and control.
Treatments:	The fish were fed once daily during the exposure period with a commercially prepared finely ground pellet food, mixed with the test material at a nominal concentration of 1000 times the EEC. Based on preliminary studies a nomi-

	nal dietary concentration of 1.47 x 10^8 cfu/g was selected for the definitive study.
Duration:	20 days
Test conditions:	The test was conducted at a target temperature of 12 ± 2 °C. Aeration was maintained constantly throughout the exposure period to maintain both a homogeneous mixture of the test material and acceptable oxygen concentrations. The photoperiod used was 16 hours light and 8 hours dark. Dissolved oxygen, pH and temperature were measured and recorded daily.
Deviations from guideline	None.
Endpoint:	No mortality was observed among fish exposed to the ABG-6305 treatment for the first 13 days. Mortality was first observed on day 14 and by day 20, 77% mortality was observed in the treatment solutions. At this stage thirty randomly selected control fish, all surviving fish and all test organisms which had died were preserved and sent for histopathological examination. At test termination external examination revealed no lesions, necroses, or tumors, which could be attributed to ABG-6305 on the skin, fins or gills. Measurement of the test fish averaged 44 ± 6.7 mm with a weight of 0.72 ± 0.24 g while control fish averaged 50 ± 4.3 mm and weighed 1.02 ± 0.22 g. Histopathological examination showed no evidence of infection or pathogenicity associated with ABG-6305 under the conditions of this study. Lesions occurred in the gills and thymus in greater incidence among treated compared to control fish, but these lesions did not appear to be primarily related to infectivity of ABG-6305. An increase in the severity of gill changes were considered non-specific and not associated with an inflammatory response. Multifocal necrosis in the thymus occurred in exposed rainbow trout and may have occurred secondary to stress.
Observations:	A 20-day exposure to ABG-6305 at treatment levels of 500x the EEC in water (nominally 7.36 x 10^{10} cfu/L) and 1000x the EEC in feed (nominally 1.47 x 10^8 cfu/g) had an adverse effect on trout survival and growth. There was no evidence of pathogenicity. Effects may have been due to or influenced by high turbidity, suspended solids and lowered pH. Turbidity levels in the test solution were 160x that of the control and this may have resulted in fish having difficulty in identifying and consuming food particles. The difference in growth between treated and control fish is believed to have been due to an adverse response to the conditions produced by the addition of ABG-6305 at nominal aqueous concentrations of 500x the EEC.

Results:

A summary of endpoints is given in the table below.

Table B.9.2.2.a: Toxicity effects/ Infectivity / Pathogenicity of the MPCA to fish

Test species	Rainbow trout (*Onchorhyncus mykiss*)
Toxicity	20 day LC_{50} /NOEC: < 7.36 x 10^{10} CFU/L (nominal) < 5.4 x 10^{10} CFU/L (mean measured)
Infectivity / Pathogenicity	No signs of infectivity and pathogenicity.

Comments and conclusion RMS:

The study was previously evaluated in the DAR (May 2007) and considered acceptable. Information found to be missing from the previous study summary added below:

Measured concentrations of test item in the aqueous exposure solutions and the food preparations were generally consistent between replicate samples (see tables 4 and 5 below). The mean measured concentration, determined in freshly prepared solutions was 5.4 x 10^{10} cfu/mL which averaged 73% of the nominal concentration. Sampling of representative aged aqueous exposure solutions following test termination (i.e., solutions samples on test day 22) provided a mean result of 6.8 x 10^{7} cfu/mL establishing a recovery of 93%. The aged test solutions were stirred well during sampling which had not been possible during the renewal exposure period since doing so would have stressed the test organisms. Comparison of the measured concentration of test item in the aqueous solutions during the in-life exposure period (minimal mixing due to the presence of the test fish) and samples removed at test termination (vigorous mixing) established that approximately 23% of the test material settled to the bottom of the exposure vessels.

Table 5. **Results of the analyses of the exposure solutions for Xentari Technical Powder (ABG-6305) concentration during the static renewal exposure of rainbow trout (*Oncorhynchus mykiss*).**

Test Day	Nominal Concentration (cfu/L)					
	7.36×10^{10}			Control		
	A	B	C	A	B	C
	Measured Concentration (cfu/mL)					
0	3.1×10^{7}	3.0×10^{7}	3.4×10^{7}	2.0×10^{1}	0	0
4	4.6×10^{7}	4.2×10^{7}	5.2×10^{7}	8.0×10^{1}	3.3×10^{1}	6.0×10^{1}
7	7.0×10^{7}	5.9×10^{7}	7.5×10^{7}	2.3×10^{1}	1.3×10^{1}	3.7×10^{1}
11	5.6×10^{7}	3.9×10^{7}	4.2×10^{7}	7.3×10^{1}	9.3×10^{1}	3.3×10^{1}
14	6.1×10^{7}	5.4×10^{7}	6.4×10^{7}	0.3×10^{1}	2.7×10^{1}	3.0×10^{1}
18	5.4×10^{7}	5.1×10^{7}	6.5×10^{7}	5.0×10^{1}	4.7×10^{1}	4.7×10^{1}
22[a]	7.3×10^{7}	6.2×10^{7}	7.0×10^{7}	3.8×10^{3}	4.3×10^{1}	5.7×10^{1}
Mean Measured Concentration[b] (cfu/mL)		5.4×10^{7} (1.4×10^{7})			NA[c]	
Mean Measured Concentration[b] (cfu/L)		5.4×10^{10} (1.4×10^{10})			NA	

[a] Samples removed following test termination (Day 20) to establish the concentration of Xentari Technical Powder (ABG-6305) in an aged exposure solution. The exposure solutions were stirred prior to sampling.
[b] Standard deviation is presented in parentheses.
[c] NA = Not Applicable.

Table 4. **Concentration of Xentari Technical Powder (ABG-6305) measured in the food administered to the rainbow trout (*Oncorhynchus mykiss*) during the static renewal exposure.**

Lot No.	Date Prepared	Test Day Analyzed	Nominal Concentration cfu/g	Measured Concentration cfu/g	% Recovery	Control Lot No.	Measured Concentration cfu/g
051393	13 May 93	[a]	1.47×10^{8}	1.5×10^{8}	99.3	052193-C	0
051393	13 May 93	6	1.47×10^{8}	1.6×10^{7}	10.6	052193-C	2.1×10^{4} (0.014%)
052893	28 May 93	7	1.47×10^{8}	1.0×10^{8}	69.4	052893-C	0
060193	01 June 93	11	1.47×10^{8}	8.2×10^{7}	55.8	060193-C	0
060393	03 June 93	13	1.47×10^{8}	1.6×10^{8}	110	060393-C	0
060793	07 June 93	17	1.47×10^{8}	1.1×10^{8}	77.6	060793-C	0

[a] This lot of food was analyzed one day prior to test initiation.

The study protocol states that the dilution water used during the definitive test should have a pH within the range of 6.9 to 7.2 and a specific conductivity within the range of 120 to 170 pmhos/cm. The dilution water used during this study had a pH range of 7.1 to 7.5 and a specific conductivity range of 11 0 to 130 pmhos/cmn.

The study protocol states that the test is to be conducted for a minimum of 30 days. Due to significant mortality observed among fish exposed to the treatment level solutions, the study was terminated on exposure day 20.

These slight deviations are not expected to have had any influence on the study results.

The most recent guideline applicable to this test is OPPTS 885.4200 (1996). As the test was performed in 1991, the predecessor of this guideline was used. This is considered acceptable, however a comparison to the current guideline was made.

At the study termination 14 out of 30 fish survived in the treatment while there were no mortalities in the control. There were significant differences, p =0.025 weight between the treated fishes (0.76 ± 0.09 g) and the control group (1.01 ± 0.06 g). Regarding the in size, there were no significant difference between the treated fish (44 ± 1.5 mm) and control group (50 ± 4.3 mm).

The report hypothesizes that effects seen are related to impaired vision due to the high turbidities in the treatments, which potentially lead to starvation of test animals. This is to a certain extent a logical explanation, although literature suggest that the feeding abilities of juvenile trout for small and larger natural live prey is not affected by high levels of turbidity (up to 160 Nephelometric Turbidity Units, NTU) in rainbow trout, as vision is not the only sense used for predation (Rowe et al. 2003[1]). On the other hand, the trout in the present study were fed dried pelleted food, which is not comparable to live wild prey as the chemical signals are different and vision could therefore play a bigger role for feeding. The turbidity levels in the treatments of this study were up to 180 NTU as compared to 0.5 NTU in the controls. Also fish can normally survive prolonged periods of starvation (up to 4 weeks for 25g juvenile rainbow trout in Sumpter et al. 1991[2]). Therefore it is questionable whether the effects on growth and survival are indeed an isolated physical effect as concluded in the previous evaluation. The cause of mortality could be a combination of starvation and exposure to the test substance or only be related to turbidity, which can however not be said with certainty with the current dataset.

Furthermore, two routes of exposure affected the test animals. By only considering aqueous exposure for the endpoint, this endpoint would be more conservative, while the exposure may have been higher, leading to an overestimation of effect. On the other hand a minimum of 23% of the test material precipitated to the bottom of the aquaria, actually lowering the direct exposure of test item to fish. Therefore, the effect may also have been underestimated.

No signs of pathogenicity or infectivity were observed. According to the study report: "*Histopathologic examination showed no evidence of infection or pathogenicity associated with the Xentari Technical Powder (ABG-6305) under the conditions of this study. Lesions occurred in the gills and the thymus in greater incidence among exposed over control rainbow trout but these lesions did not appear to be primarily related to infectivity of the Xentari Technical Powder (ABG-6305). An increase in incidence*

[1] Rowe DK, Dean TL, Williams E, Smith J. Effects of turbidity on the ability of juvenile rainbow trout, *Oncorhynchus mykiss*, to feed on limnetic and benthic prey in laboratory tanks. New Zealand Journal of Marine and freshwater research,2003, Vol 37, Issue 1.

[2] Sumpter JP, Le Bail PY, Pickering AD, Pottinger TG, Carragher JF. The Effect of starvation on growth and plasma growth hormone concentrations of rainbow trout, *Oncorhynchus mykiss*. Gen Comp Endocrinol. 1991;83:94–102.

and severity of gill changes (fusion of gill lamellae and hyperplasia of gill epithelium) were considered nonspecific and not associated with an inflammatory response. Multifocal necrosis in the thymus occurred in exposed rainbow trout and may have occurred secondary to stress". No stress symptoms were reported, dark pigmentation and other physiological or behavioral parameters. There were no reports that the target tissues were affected resulting in cell disruption and septicemia. Therefore, the effect is likely not a direct consequence of the microorganism itself, of the metabolites or Cry proteins but as a result of the high turbidity.

The study is considered relevant and reliable with restriction. As there were no signs of infectivity and pathogenicity observed and the endpoint represents most likely a worse-case scenario it can be used for risk assessment.

Reference 8.2.1/03

Reference:	[redacted] (1995a) Xentari technical powder (ABG-6305) – toxicity to rainbow trout (*Oncorhynchus mykiss*) during a 30-day static renewal test. Unpublished report No.: 95-7-5977
Guideline:	FIFRA Guideline 154A-19.
GLP:	Yes (40 CFR Part 160)
Material and methods:	Test material: ABG-6305. Lot No 95-509-BD. *Bacillus thuringiensis* subsp. *aizawai*, strain ABTS-1857 (spore count 5.6 x 10^{10} cfu/g).A 30-day semi-static test system with renewal was used to determine the toxicity (LC_{50}) to rainbow trout of continuous exposure to ABG-6305 at aqueous and dietary concentrations equivalent to 1000 times expected environmental concentrations (EEC). The test vessels (glass aquaria) were filled with approximately 18 L volume of test media. Dilution water was recirculated at a rate sufficient to maintain test material suspension while avoiding undue stress to the exposed fish. All replicate treatment level and control solutions were renewed every 72 hours. For the aqueous administration a stock suspension of 460 mg/L was prepared by mixing 34.5 g of ABG-6305 with 1.5 L of dilution water. This initial stock solution was then diluted to 75 L with dilution water giving a nominal concentration of 460 mg/L and after mixing 18 L was transferred to each replicate test vessel. Appropriate dilutions were used for the decreasing range of concentrations tested. Based on the results of an initial range-finding study, nominal aqueous concentrations of 15, 60, 130, 250, 320, 390 and 460 mg ABG-6305-BD per litre were selected for the definitive study. An attenuated control was used containing an irradiated sample of ABG-6305 Lot no 95-509-BD (spore count <10^2 cfu/g). The attenuated control was prepared by mixing 11.5g of the test material with 0.5

	L of dilution water. This initial stock solution was then diluted to 25 L of dilution water resulting in a nominal concentration of 460 mg/L. The attenuated control was mixed vigorously before delivery of 18 L to the appropriately labelled aquaria. One control aquaria was also maintained during the study, containing 18 L of dilution water only. Seventy rainbow trout were impartially selected and equally distributed among the test and control aquaria: 10 organisms per replicate vessel, 30 organisms per treatment and control. The loading density during the toxicity test was 0.34 g biomass/L. The fish were fed once daily during the exposure period with a commercially prepared finely ground pellet food, mixed with the test material at a nominal concentration of 1000 times the EEC. Based on preliminary studies a nominal dietary concentration of 1.47 x 10^8 cfu/g or 0.736 g of ABG-6305-BD per kilogram of food was selected for the definitive study. An attenuated control was prepared using 0.736 g/kg using the irradiated material. The test was conducted at a target temperature of 12 $\pm$ 2 °C. Aeration was maintained constantly throughout the exposure period to maintain and acceptable oxygen concentrations. The photoperiod used was 16 hours light and 8 hours dark. Dissolved oxygen, pH and temperature were measured and recorded daily in each vessel. At renewal period these water quality parameters were measured in both the freshly prepared and aged test solutions. Observations of mortality were made once daily. Due to the cloudiness of the exposure solutions, a fine mesh net was drawn through the solutions daily in an effort to observe mortalities. Mortalities were confirmed on renewal days. Fish that died were removed from the test vessels. Observation on the physical characteristics of the test solution (e.g. precipitate) were also made and recorded daily. Measurement of colony forming units /L in the test solution, media control and attenuated control were performed. Selected exposure solutions were sampled on days 0, 3, 6, 9, 12, 15, 21 and 24. For the duration of the definitive test, concentration verification of each batch of the amended food mix and each batch of the control food was routinely conducted. In this study the LC_{50} value is based on adjusted nominal concentrations. Adjusted nominal concentrations were calculated using rounded (two significant figures) cfu determinations.
Test substance	XenTari technical powder (ABG-6305), *Bacillus thuringiensis* subsp. *aizawai*, strain ABTS-1857. Lot no.: 95-509-BD, Spore count 5.6 x 10^{10} CFU/g
Test species:	Rainbow trout (*Oncorhynchus mykiss*)
Number of test animals:	Seventy rainbow trout were impartially selected and equally distributed among the test and control aquaria: 10 organisms per replicate vessel, 30

	organisms per treatment and control.
Treatments:	The fish were fed once daily during the exposure period with a commercially prepared finely ground pellet food, mixed with the test material at a nominal concentration of 1000 times the EEC (to achieve the required 100 times the EEC). Based on preliminary studies a nominal dietary concentration of 1.47 x 10^8 cfu/g or 0.736 g of ABG-6305-BD per kilogram of food was selected for the definitive study. An attenuated control was prepared using 0.736 g/kg using the irradiated material.
Duration:	30 days
Test conditions:	The test was conducted at a target temperature of 12 ± 2 °C. Aeration was maintained constantly throughout the exposure period to maintain and acceptable oxygen concentrations. The photoperiod used was 16 hours light and 8 hours dark. Dissolved oxygen, pH and temperature were measured and recorded daily in each vessel.
Deviations from guideline	None
Endpoint:	No mortality was observed in fish exposed to adjusted nominal aqueous concentrations of 13 mg/L. Mortality of 40%, 20%, 40% and 30% were observed following 30 days exposure in adjusted nominal aqueous concentrations of 61, 110, 240 and 320 mg/L respectively. Mortality of 60% and 70% were observed in the adjusted nominal aqueous concentrations of 370 and 440 mg/L, respectively at test termination. Mortality of 100% was observed among the rainbow trout exposed to the attenuated control at 4 days. Mortality of 10% was observed among organisms exposed to the negative control following 30-days.
Observations:	Based on this study, no trout mortality was attained at adjusted nominal aqueous concentrations of 13 mg/L and 70% mortality at 440 mg/L, following 30 days exposure. The 30-day LC50 on adjusted nominal concentration was 310 mg/L (corresponding to 95% confidence limits of 170 to 1160 mg/L).

Results:

A summary of endpoints is given in the table below.

Table B.9.2.2.a: Toxicity effects/ Infectivity / Pathogenicity of the MPCA to fish

Test species	Rainbow trout (*Onchorhyncus mykiss*)
Toxicity	30 day LC_{50} : 1.74 x 10^{10} CFU/L (mean measured)
Infectivity / Pathogenicity	Pathogenicity or infectivity were not studied.

Comments and conclusion RMS:

The study was previously evaluated in the DAR (May 2007) and considered acceptable. Additions to the previous study evaluation are made below.

The verifications of exposure were performed using a suitable plate count assay and results were expressed in CFU/L or CFU/g food.

The measured concentrations in this study were 13, 61, 110, 240, 320, 370 and 440 mg/L (nominal was 15, 60, 130, 250, 320, 390 and 460 mg/l).

The most recent guideline applicable to this test is OPPTS 885.4200 (1996). As the test was performed in 1991, the predecessor of this guideline was used. This is considered acceptable, however a comparison to the current guideline was made.

The RMS translated the LC50 of 310 mg test item/L in 1.74 x 10^{10} CFU/L, based on the specification of the test item (5.6 x 10^{10} CFU/g).

The attenuated control lead to 100% mortality after 4 days in contrast to the spore treatments where maximum 70% mortality was observed within the 30 day test period. This seems to indicate that substances present in the fermentation broth are highly toxic to fish. The report does not give any further explanation as to why the attenuated control showed such high mortality. Exposure to the level applied to the attenuated control is not expected to occur in the environment. Therefore this finding is not relevant for risk assessment directly, but it shows that a part of the toxicity can be attributed to the fermentation broth.

Temperature shows slight deviations and higher variability from the optimal values for rainbow trout (12 ± 2°C instead of 10 ± 1.5°C), while oxygen was constantly above 60%, hardness and alkalinity were constant and the pH varied very little across replicates and treatments (0.4 units among controls, maximal 1 unit across treatments). The slightly higher variation in temperature is not expected to have had an effect on the results. Therefore, the water quality parameters are not considered to have influenced the study results.

Pathogenicity or infectivity were not studied.

The study is considered relevant and reliable and the endpoint can be used in risk assessment.

Toxin/metabolite from microbial pest control agent (MPCA)

No study or information was submitted. According to the information provided in the former DAR: "*Bacillus thuringiensis* subsp. *aizawai,* Strain ABTS-1857, like other Bt strains commercially available, has been shown not to contain β-exotoxins or enterotoxins. Strict maintenance of environmental conditions and quality control analysis during the manufacturing process ensures the absence of potential microbial and non-microbial contaminants or potential animal or human pathogens".

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.

Considering the mode of action, it is not expected that these host-specific toxins will be active in the fish gastrointestinal tract as they will be inactivated by the protease enzymes (please refer also to the Vol. 3 B6).

B.9.2.3 Infectiveness to fish

Infectiveness of *Bacillus thuringiensis* ssp *aizawai* ABTS-1857 to fish was studied in Collins 1993 (8.2.1/02), a 20 day static renewal test and no signs of infectiveness were found.

B.9.2.4 Pathogenicity to fish

Pathogenicity of *Bacillus thuringiensis* ssp *aizawai* ABTS-1857 to fish was studied in Boeri 1991a (8.2.1/01) and Collins 1993 (8.2.1/02). While the study duration in Boeri 1991a was considered too short to detect pathogenicity, the study of Collins 1993, a 20 day semi static renewal test with rainbow trout did not detect any signs of pathogenicity.

B.9.2.5 Effects on freshwater invertebrates

B.9.2.6 Toxicity to freshwater invertebrates

Microbial pest control agent (MPCA)

Reference 8.2.2/01

Reference:	M.K. Collins (1995b) - ABG-6305 – Toxicity to water fleas (Daphnia magna) under static renewal conditions. Springborn Laboratories Inc., Unpublished report No.: 95-5-5855
Guideline:	Based on US EPA Assessment Guidelines (Subdivision M; Section 154A-20. US EPA 1989)
GLP:	Yes (40 CFR Part 160)
Material and methods:	Test material: ABG-6305. Lot No 95-509-BD. *Bacillus thuringiensis* var. *aizawai*, strain ABTS-1857 (potency 28151 IU/mg, 2 x 10^{11} cfu/g). The toxicity of ABG-6305 to *Daphnia magna* (≤12 to 24 hours old) was determined in a 10-day semi-static study conducted at 20 ±1°C. Media renewal was carried out every 48 hours. The test compartments were stirred 2-L glass vessels that contained 1000ml of test suspension, placed in a temperature-controlled waterbath. Daphnids were housed within two retention chambers that were suspended below the surface of the exposed suspension within each glass vessel. The retention chambers were constructed of 5-cm diameter, 8-cm high glass jars with the bottoms removed and replaced with Nitex® screen. This screen allowed the Daphnids to be exposed to a continuous flow of test material. The chambers were oscillated twice daily to avoid accumulation of undissolved test material. Two replicate vessels were maintained for each concentration of test material and the control. Test suspensions were aerated throughout the exposure period. The photoperiod used was 16 hours light and 8 hours dark. Definitive test concentrations were based on a 10-day screening test. For the definitive test nominal concentrations of ABG-6305 used were 0 (control), 1.9, 3.2, 5.4, 9.0, 15, 25 and 42 mg/L. Ten Daphnia were randomly distributed into replicate vessels for each treatment (20 organisms per treatment level and control). At renewal all surviving Daphnids were transferred into newly prepared test solutions. The Daphnids were fed daily. Observations for mortalities were made daily and any organisms that were considered dead were examined for heartbeat. Observations of the physical characteristics of the test suspension (e.g. cloudiness) were made and recorded daily. Dissolved oxygen, pH and temperature were measured daily in one replicate vessel of the control(s) and for each concentration. Total hardness and conductivity were measured for each batch of dilution water. Determination of viable colony forming units were made using a plate count technique. One replicate vessel of each exposure suspension was sampled on days 0, 2, 4, and 6. This sampling scheme encompassed two exposure periods including freshly prepared suspensions and aged suspensions.

	These exposure periods represented the longest period of time between suspension renewals.
Micro-organism	XenTari technical Powder (comprised of *Bacillus thuringiensis* var *aizawai*, strain ABTS-1857. ABG-6305), Lot no. 95-509-BD
Test species:	Water fleas (Daphnia magna)
Number of test animals:	Ten Daphnia were randomly distributed into replicate vessels for each treatment (20 organisms per treatment level and control).
Treatments:	Nominal concentrations of ABG-6305 used were 0 (control), 1.9, 3.2, 5.4, 9.0, 15, 25 and 42 mg/L
Duration:	10 days
Test conditions:	20 ± 1°C; photoperiod used was 16 hours light and 8 hours dark.
Deviations from guideline	Routine water and food contamination screening analysis for pesticides, PCB's and metals and turbidity analysis were conducted using standard US EPA procedures by Lancaster Laboratories, Lancaster, PA
Endpoint:	After 9 days exposure, 100% mortality was observed in the daphnids exposed to the highest concentration tested (34 mg/L). At test termination (day 10), mortality of 0, 30 and 95% was observed among Daphnids exposed to the 5.9, 10 and 20 mg/L treatment levels, respectively. The 10-day LC_{50} value for ABG-6305 and Daphnia magna was calculated by probit analysis to be 12 mg/L (95% confidence interval was 10 to 14 mg/L).
Observations:	The 10-day LC_{50} value for ABG-6305 and Daphnia magna was calculated to be 12 mg/L (95% confidence interval - 10 to 14 mg/L).

Results:

A summary of endpoints is given in the table below.

Table B.9.2.6.a: Toxicity effects / Infectivity / Pathogenicity of the MPCA to freshwater invertebrates

Test species	*Daphnia magna*
Toxicity	10-day LC_{50} (survival): 2.4 x 10^8 CFU/L (mean measured concentration)
Infectivity / Pathogenicity	Not tested.

Comments and conclusion RMS:

The study was previously evaluated in the DAR from May 2007 considered acceptable. Additions and changes made to the previous study evaluation are made below:

The temperature ranged between 20 and 22°C, while oxygen levels ranged from 8.1 - 9.3 mg/L and pH from 7.9 – 8.3, hardness ranged from 170-180 mg/L and alkalinity was 110 mg/L. As all water quality parameters were within the acceptable ranges and therefore they are not expected to have had an influence on the results.

Measured concentrations of test item used were calculated to be 0 (control), 1.1, 2.0, 3.4, 5.9, 10, 20 and 34 mg/L. The MPCA caused effects on survival of daphnids. According to the Table 3 of the study report, at 20 and 34 mg/L , exposure suspensions were observed to be slightly cloudy throughout the exposure period. The previous RMS translated the measured LC50 of 12 mg test item/L from the study into 2.4 x 10^8 CFU/L, based on the technical specification of 2 x 10^{11} CFU/g given in the previous evaluation. The study is considered relevant and reliable. The results and endpoints as reported above can be used in risk assessment.

Reference 8.2.2/02

Reference:	R.L. Boeri (1991b) Chronic toxicity of ABG-6305 to the daphnid Daphnia magna Unpublished report, report number 90162-A
Guideline:	U.S. EPA. Hazard Evaluation Division Standard Evaluation Procedure. Daphnia magna Life-cycle (21-day renewal) Chronic Toxicity Test (1985) U.S. EPA. Pesticide Assessment Guidelines. Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms 72-4. Fish Early Life Stage and Aquatic Invertebrate Life-Cycle Studies. Ecological Effects Branch, Hazard Evaluation division, Office of Pesticide Programs (1988)
GLP:	Yes (40 CFR Part 160)
Material and methods:	Test material: ABG-6305. Lot No 42-221-BD. Bacillus thuringiensis subsp. aizawai, strain ABTS-1857 (potency 28151 IU/mg, 2 x 10 ^{11} cfu/g). The chronic toxicity of ABG-6305 to *Daphnia magna* (<24 hours old) was determined in a 21-day replacement study conducted at 20 ± 2°C. Media renewal was carried out on days 2, 4, 7, 9, 12, 14, 16 and 18. The test compartments were 250 ml glass beakers, placed within an incubator. The photoperiod used was 16 hours light and 8 hours dark. Aeration was not required to maintain dissolved oxygen concentrations above acceptable levels.

	Definitive test concentrations were based on a 72-hour screening test. The stock suspension of test material was prepared immediately prior to addition of the test organisms and before each renewal. 0.5 g of ABG-6305 was combined with 1L of dilution water to produce a nominal concentration of 500 mg/L. Appropriate concentrations of this stock solution were then added directly to each exposure vessel. Nominal concentrations of ABG-6305 used were 0 (control), 0.5, 5 and 74 mg/L. Twenty-seven Daphnia were randomly distributed among 11 replicates of each treatment. Seven replicates each contained a single Daphnid to collect survival, growth and reproduction data. Four replicates contained 5 daphnids for survival data only. Observations for mortalities and immobile adult D. magna were made daily, juveniles were counted and removed daily. Dead organisms were removed when first observed. At the end of the exposure period all surviving adult Daphnia from each exposed vessel that contained a single test organism were weighed (dry weight determined). Test substance concentration was determined on pooled samples from each tested concentration and control. Samples were collected for testing on the day the test was initiated and on days 2, 4, 7, 9, 11, 14, 16 and 18. Dissolved oxygen, pH, conductivity and temperature were measured daily in one test chamber for each concentration.
Test substance	ABG-6305 technical powder biological insecticide, Lot no. 42-221-BD, (comprised of *Bacillus thuringiensis* var *aizawai*, strain ABTS-1857). Spore count 2 x 10^{11} CFU/g
Test species:	Daphnia magna
Number of test animals:	Twenty-seven Daphnia were randomly distributed among 11 replicates of each treatment. Seven replicates each contained a single Daphnid to collect survival, growth and reproduction data. Four replicates contained 5 daphnids for survival data only.
Treatments:	Nominal concentrations of ABG-6305 used were 0 (control), 0.5, 5 and 74 mg/L
Duration:	21-days
Test conditions:	20 ± 2°C; the photoperiod used was 16 hours light and 8 hours dark. Aeration was not required to maintain dissolved oxygen concentrations above acceptable levels.
Endpoint:	Control survival at 21 days after test initiation was at least 95%. Water quality parameters were within acceptable limits throughout the test. Survival of the first generation Daphnids exposed to 5 mg/L and 74 mg/L ABG-6305 were both significantly different compared to the control. Survival of the first generation, offspring production, weight, and the occurrence of sublethal effects

	were not significantly different between the 0.5 mg/L treatment and control. Production of young by the first generation Daphnids was first observed on day 10 in test vessels containing 0.5 and 5.0 mg/L ABG-6305 and the control. Daphnids exposed to 74 mg/L were killed prior to sexual maturity. Results were interpreted by standard statistical techniques.
Observations:	Exposure of Daphnia magna to ABG-6305 resulted in a no observable effect concentration (NOEC) of 0.5 mg/L, a lowest observable effect concentration (LOEC) of 5.0 mg/L and a maximum acceptable toxicant concentration (MATC) of 1.6 mg/L.

A summary of endpoints is given in the table below.

Table B.9.2.6.a: Toxicity effects / Infectivity / Pathogenicity of the MPCA to freshwater invertebrates

Test species	*Daphnia magna*
Toxicity	21-day NOEC (survival): 10^9 CFU/L (nominal) and 1.94 x 10^8 CFU/L (mean measured) 21-day NOEC (reproduction): 10^8 CFU/L (nominal), 1.43 x 10^7 CFU/L (mean measured)
Infectivity / Pathogenicity	Not tested.

Comments and conclusion RMS:

The study was previously evaluated in the DAR of May 2007 and considered acceptable. Additions and changes to the previous study evaluation are made below.

Nominal concentrations of ABG-6305 used were 0 (control), 0.5, 5 and 74 mg/L corresponding to 10^5, 10^6, 14.8 x 10^6 spores/mL. Mean measured concentrations in CFUs were 1.43 x 10^4, 1.94 x 10^5, 4.0 x 10^6 CFU/ mL (1.43 x 10^7, 1.94 x 10^8, 4.0 x 10^9 CFU/L).

As water quality parameters were in the ranges known to be required for Daphnia testing throughout the study they are not considered to have had an influence on the results. According to the study report the test media at the two highest concentrations of 5 and 74 mg/L exhibited insoluble test material throughout the test.

The most recent guideline applicable to this test is OPPTS 885.4240 (1996). As the test was performed in 1991, the predecessor of this guideline was used. This is considered acceptable, however a comparison to the current guideline was made.

The MPCA caused effects on survival, reproduction and growth in Daphnids. The 21-d NOEC for reproduction is determined to be 0.5 mg test item/L, which translates into 10^8 CFU/L (nominal) and 1.43 x 10^7 CFU/L (mean measured). Survival was affected at the highest concentration and therefore the 21d NOEC for survival was found to be 5 mg/L (nominal), which translates into 10^9 CFU/L (nominal) and 1.94 x 10^8 CFU/L of mean measured exposure.

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

Reference 8.2.2/03

Reference:	Ward, T.J., Kowalski, P.L., Boeri, R.L. (1993) Acute Toxicity of Components of ABG-6305 to the Daphnid Daphnia magna - addendum to MRID 41974802 Unpublished report, Wilbury Laboratories Inc., Report Nr. 123-AB
Guideline:	U.S. EPA. Standard Evaluation Procedure. Acute Toxicity Test for Freshwater Invertebrates. Hazard Evaluation Division. (1985) U.S. EPA. Pesticide Assessment Guidelines. Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms 72-4. Fish Early Life Stage and Aquatic Invertebrate Life-Cycle Studies. Ecological Effects Branch, Hazard Evaluation division, Office of Pesticide Programs (1988)
GLP:	Yes (40 CFR Part 160)
Material and methods:	Test materials: Technical Powder, Lot 71-951-W5, Technical Pellet, Lot 71-951-W5-TP, Technical Supernatant, Lot 71-951-W5-TS, Technical Supernatant Heated, Lot 71-951-W5-TSH and Spore/Crystal Preparation Batch 382-SC (all samples derived from a single fermentation batch: 382). The toxicity of a single concentration of ABG-6305 and its components to Daphnia magna (<24 hours old) was determined in a 10-day static renewal study conducted at 20 ± 1°C. Media renewal was on day 2, 4, 7 and 9. The aim of the test was to determine which component(s) of the XenTari Technical Powder was responsible for previously observed toxicity. The technical powder was prepared from the fermentation batch in a manner identical to the commercial process. Technical powder supernatant and pellet fractions were prepared by washing a 100g sample of the technical powder five times by suspension in deionised water and centrifuged at a RCF of 17000 times gravity. This RCF is sufficient to pellet both spores and crystals, yielding a cell free fraction. All supernatant volumes were mixed and lyophilized to a dry pellet. A second sample of technical powder supernatant was autoclaved for 15 minutes to evaluate the heat labile fraction. The spore / crystal fraction was derived directly from fermentation 382 harvest beer. A

	pellet was prepared as above and washed 5X with water to remove particulates. The test fractions were normalised to be equivalent to a specified amount of technical powder. The stock suspension of test materials was prepared immediately prior to addition of the test organisms and before each renewal. 0.6 g of spore / crystal preparation, 0.6 g of technical supernatant, 0.6 g of technical pellet, 0.6 g of technical supernatant heated and 1.0 g of technical powder were individually diluted in 1000 ml water to produce 600 mg/L of spore/crystal preparation, technical supernatant, technical pellet and technical supernatant heated and 100 mg/L of technical powder. The stock solutions were mixed thoroughly and appropriate amounts added directly to the test vessels. The test compartments were 250 ml glass beakers containing 200ml of test solution randomly placed within an incubator. The photoperiod used was 16 hours light and 8 hours dark. Gentle aeration was employed in each vessel throughout the test. Dissolved oxygen, pH, conductivity and temperature were measured and recorded daily in each test chamber that contained live animals. Definitive test concentrations were based on historical data rather than on a screening test. Twenty Daphnia were randomly distributed among 4 replicates of each treatment. Observations for mortalities and sublethal effects (immobilization and lethargy) were made daily. Control Daphnids had an average weight (blotted dry) of 0.8 mg, resulting in a loading rate during the toxicity testing of 0.020 g/L.
Test substance	XenTari technical powder (ABG-6305), Lot no.: 71-951-W5, (comprised of Bacillus thuringiensis var aizawai, strain ABTS-1857) Spore count 1.76 x 10^{11} CFU/g
Test species:	*Daphnia magna*
Number of test animals:	Twenty Daphnia were randomly distributed among 4 replicates of each treatment
Treatments:	0.6 g of spore / crystal preparation, 0.6 g of technical supernatant, 0.6 g of technical pellet, 0.6 g of technical supernatant heated and 1.0 g of technical powder were individually diluted in 1000 ml water to produce 600 mg/L of spore/crystal preparation, technical supernatant, technical pellet and technical supernatant heated and 100 mg/L of technical powder.
Duration:	10-days
Test conditions:	20 ± 1°C; the photoperiod used was 16 hours light and 8 hours dark.
Endpoint:	Results of the toxicity testing are given in Tables 1 (survival and effects) and

	2 (time to effect for 50% of the organisms (ET50) from toxicity testing).

Table 1

Survival and effects data for *Daphnia magna* exposed to ABG-6305 components

Test Substance	Concentration (mg/L)	48-hour		10-day	
		Live	Unaffected	Live	Unaffected
Control	0	100%	100%	90%	90%
Spore / Crystal Preparation	600	100%	100%	5%	0%
Technical Supernatant	600	100%	100%	0%	0%
Technical Pellet	600	45%	45%	0%	0%
Technical Supernatant Heated	600	100%	100%	25%	0%
Technical Powder	100	85%	85%	0%	0%

Table 2

Time to effect for 50% of the organisms (ET_{50})

Test Substance	ET_{50} (hours)	95% CI (hours)
Spore / Crystal Preparation	207	195-207
Technical Supernatant	83	77-90
Technical Pellet	24	Could not be calculated
Technical Supernatant Heated	196	190-203
Technical Powder	68	58-80

Observations:	The toxic effects of the technical powder are produced by a combination of both supernatant and the pellet. The supernatant toxicity to Daphnia magna is reduced by heating. The spore-crystal complex does not appear to contribute to the toxicity of the pellet.

A summary of endpoints is given in the table below.

Table B.9.2.6.a: Toxicity effects / Infectivity / Pathogenicity of the MPCA to freshwater invertebrates

Test species	*Daphnia magna*

Toxicity	Not applicable. Ten day toxicity test aimed at identifiying the components causing the toxicity seen for Daphnia in earlier studies. It concluded that both the supernatant and the pellet contribute to the toxicity, while the onset of toxicity differed somewhat between the different components. The Technical Pellet had the fasted onset of toxicty (ET50 24h), whereas the spore/crystal fraction (ET50 270h) and the Heated Technical Supernatant (ET50 196h) had the longest.
Infectivity / Pathogenicity	Not tested.

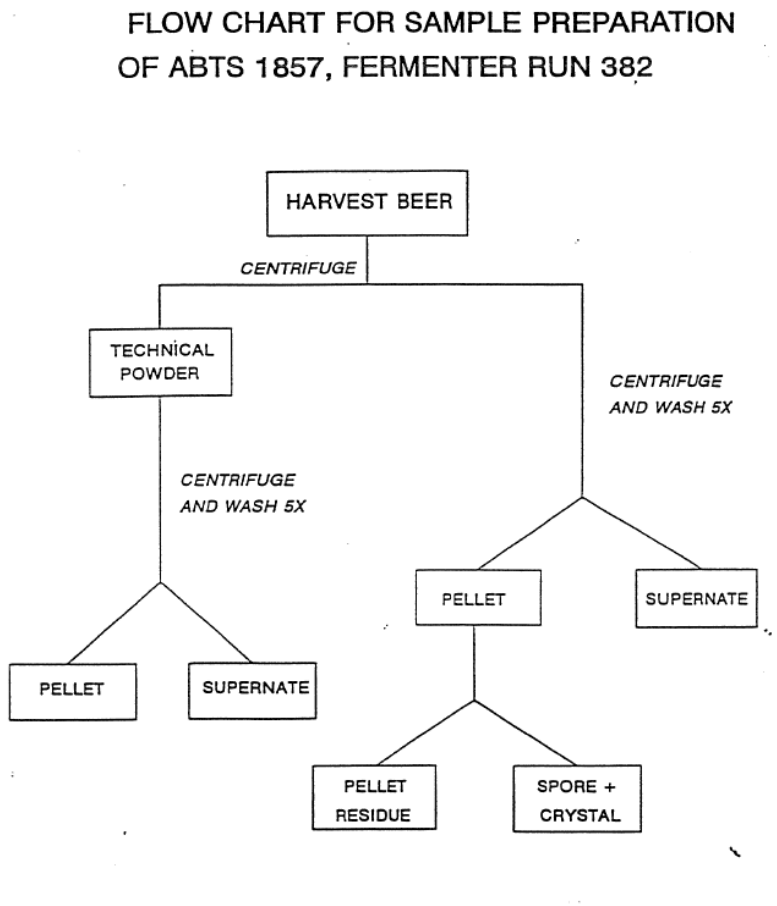
Comments and conclusion RMS:

The study was previously evaluated in the DAR of May 2007 and considered acceptable. Additions and changes to the previous study evaluation are made below.

Statistical analysis of the data in the study report was carried out by an external expert.

During the test pH ranged from 8.0 to 8.5, the mean temperature was 20.4 (19.5 – 20.8)°C, dissolved oxygen levels ranged between 8.1 and 9.2 mg/L, hardness between 164 and 176 mg/L and conductivity ranged between 560 to 710 µmhos/cm (= µS/cm). The water quality parameters are acceptable, although the conductivity is relatively high (recommended OECD maximum 10 µS/cm) it is still well in the ranges for freshwater (up to 500 mS/cm). As the control treatment showed 90% survival the water quality parameters are not expected to have influenced the results of this study. The conditions under which the test was conducted are acceptable.

Supplemental to the description of the derivation of the different test components, the following flow chart (note: it does not illustrate the parallel heat treated sample of technical powder supernatant) is added from the study report for better understanding:



The technical powder pellet, the technical powder supernatant, the technical powder supernatant heat treated and the spore/crystal complex were applied at 600 mg/L, while the technical powder was applied at 100 mg/L. This is the consequence of the normalization procedure, according to the dilution of the washed technical powder pellet suitable for quantitative transfer, which was 6x that of the technical powder itself.

According to the study report turbidity and suspended particles were observed in all samples, "*Insoluble material (spore/crystal Preparation was turbid with clumps of material on the bottom when aeration was stopped, Technical Supernatent had fine suspended particles, Technical pellets and Technical Powder had turbidity and particulates suspended and on the bottom of test vessels when aeration was stopped, and Technical supernatent Heated had faint turbidity) was noted in all non-control vessels throughout the test".*

All 5 components of the technical MPCA caused effects on daphnids at the concentrations tested. The effect was 100% (affected) in all treatments, the only difference seen was in terms of onset of toxicity. The Technical Pellet had the fasted onset of toxicity (ET50 24h), whereas the spore/crystal fraction (ET50 270h) and the Heated Technical Supernatant (ET50 196h) had the longest. Heat treatment of the supernatant delayed the onset of toxicity somewhat, but did not prevent it. Therefore, it must be concluded that the components toxic to daphnids are present in the technical powder compartments: technical pellet, and the supernatant. A small fraction of the toxic components may be heat labile.

In the previous study summary the following statement was made under 'Observations': 'The spore-crystal complex does not appear to contribute to the toxicity of the pellet.' The RMS agrees that this conclusion is true in terms of being derived from the big difference in the onset of toxicity for the pellet and the crystal/spore complex, but not for the technical powder and the supernatant and also not for the survival after 10 days.

The present study does not allow to derive differences between the components or allow conclusions about the origin of toxicity.

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

Reference 8.2.2/04

Reference:	M.K. Collins (1995c) Comparative toxicity test exposing Daphnids (Daphnia magna) to XenTari technical powder produced by two different recovery processes Unpublished report, Springborn Laboratories, Inc, Report Nr. 95-2-5717
Guideline:	U.S. EPA. Pesticide Assessment Guidelines Subdivision M; Section 154A-20 (1989)
GLP:	Yes (40 CFR, Part 160)
Material and methods:	Test material: Samples of XenTari technical powder, Bacillus thuringiensis var. aizawai, strain ABTS-1857, produced by two different recovery processes ABG-6305 (standard process) Lot Nos 95-508-BD (fermentor 1) and 95-509-BD (fermentor 2); and ABG-6346 (alternate process) Lot Nos 95-505-BD (fermentor 1) and 95-506-BD (fermentor 2). The purpose of this study was to compare the acute toxicity of XenTari Technical Powder, produced by two different recovery processes (centrifugation plus evaporation versus evaporation only) from each of two different fermentors, to Daphnia magna under semi-static conditions. Daphnia (≤ 24 hours old) were exposed to the test substances for 8 days with media renewal being carried out on days 2, 4 and 7. The test compartments were stirred 2-L glass vessels that contained 1000 mL of test suspension. Each test vessel contained a Teflon® coated stir bar and was placed on a magnetic stir plate, which continuously mixed the test suspension during exposure. Daphnids were housed within two retention chambers that were suspended below the surface of the exposed suspension within each glass vessel. The retention chambers were constructed of 5-cm diameter, 8-cm high glass jars with the bottoms removed and replaced with Nitex® screen. This screen allowed the Daphnids to be exposed to a

	continuous flow of test material. The chambers were oscillated twice daily to avoid accumulation of undissolved test material. Two replicate vessels were maintained for each concentration of test material and the control. Test suspensions were mixed and aerated throughout the exposure period. The photoperiod used was 16 hours light and 8 hours dark. Definitive test concentrations were based on previous studies. A nominal concentrations of 70 mg/L for each test material was prepared by adding 0.2100 g of the test material directly into 3.0 L of dilution water. The resultant test suspension was then divided between three replicate vessels. One set of dilution water control vessels were also established. Five Daphnia were randomly distributed into each retention chamber (30 organisms per treatment level and control). At renewal all surviving Daphnids were transferred into respective newly prepared test solution. Daphnids were fed daily. Observations for mortalities were made daily and any organisms that were considered dead were examined for heartbeat. Observations of the physical characteristics of the test suspension were made and recorded daily. Dissolved oxygen, pH and temperature were measured daily in the test concentration and the control using alternating replicates. Determination of viable colony forming units was made using a plate count technique for test suspensions of one lot of ABG-6305 and one lot of ABG-6346 as well as the control solution. Replicate vessels of each exposure suspension were sampled on days 0, 2 and 4. Samples were heat shocked, homogenised, sonicated and plated onto TSA using a pour plate technique. Plates were incubated at 30°C for approximately 24 to 40 hours before being read manually.
Test substance	XenTari technical powder (ABG 6305, comprised of Bacillus thuringiensis var aizawai, strain ABTS-1857), lot nos. 95-508-BD and 95-509-BD and (ABG 6346) lot nos. 95-505-BD and 95-506-BD
Test species:	*Daphnia magna*
Number of test animals:	Five Daphnia were randomly distributed into each retention chamber (30 organisms per treatment level and control).
Treatments:	A nominal concentration of 70 mg/L for each test material was prepared by adding 0.2100 g of the test material directly into 3.0 L of dilution water. The resultant test suspension was then divided between three replicate vessels. The acute toxicity of XenTari Technical Powder, produced by two different recovery processes (centrifugation plus evaporation versus evaporation only) from each of two different fermentors, to daphnia magna under semi-static conditions
Duration:	7 days

Test conditions:	19-22 °C. The photoperiod used was 16 hours light and 8 hours dark.
Endpoint:	Mortality was first observed among daphnids exposed to both standard and alternate processed technical powder prepared from fermentor one on exposure day 2 (10% and 27% respectively) with 100% mortality observed on day 7 for both standard and alternate process material. For the fermentor two prepared material mortality of daphnids was observed for standard processed material and alternate process material on day 3 (10%) and day 2 (20%) respectively. 100% mortality was observed among the daphnids exposed to the standard processed material on day 8, while 100% mortality was observed among the daphnids exposed to the alternative processed material on day 5. Throughout the 8-day period, no mortalities were observed among the control population. Results are summarized in the Table 6 below.
Observations:	The results establish that the toxicity of XenTari Technical powder was comparable between fermentation processes (standard vs. alternate) and between fermentors (fermentor 1 vs. fermentor 2). For each fermentation process and each fermentor, toxicity was first observed two days following exposure. A 100% mortality of the exposed test organisms occurred by test day 6 for the alternate process and test day 8 for the standard process.

Table 6. Summary of the mean cumulative percent mortality of daphnids (*Daphnia magna*) determined during the 8-day static renewal exposure to 70 mg/L of standard process technical powder (ABG 6305, Lots 95-508-BD and 95-509-BD) and alternate process technical powder (ABG 6346, Lots 95-505-BD and 95-506-BD) prepared from two fermentors.

	Mean Percent Mortality Exposure Day								
Treatment	0	1	2	3	4	5	6	7	8
Control Mean	0	0	0	0	0	0	0	0	0
Standard Process Mean	0	0	5	30	79	95	97	99	100
Alternate Process Mean	0	0	24	67	95	99	100	—[b]	—

[b] Observations discontinued due to previous 100% mortality.

A summary of endpoints is given in the table below.

Table B.9.2.6.a: Toxicity effects / Infectivity / Pathogenicity of the MPCA to freshwater invertebrates

Test species	*Daphnia magna*

Toxicity	Comparison of 2 fermentation recovery methods in 2 fermenters in terms of their impact on daphnia survival at 70 mg technical material/L. 100% mortality was reached within 6 - 8 days. No difference in results observed to the acute and chronic daphnia studies reported above.
Infectivity / Pathogenicity	Not tested.

Comments and conclusion RMS:

The study was previously evaluated in the DAR of May 2007 and considered acceptable. A couple of editorial changes to the previous study evaluation were made.

The study aimed at comparing the toxicity of XenTari technical powder produced by two different fermentation processes (centrifugation + evaporation versus evaporation only) to daphnids. According to the study authors, throughout the exposure, all test suspensions were observed to be cloudy. The results reveal that the two processes lead to a technical material that has comparable toxicity and significant adverse effects on daphnids. The 'evaporation only' material reached the 100% mortality 2 days earlier than the material where some of the supernatant was evaporated in advance . Also the onset of toxicity might have been slightly earlier, although this is not consistent among replicates. This difference could be explained by removing a significant part of toxic components in the fermentation broth from the technical material by applying centrifugation, so that not only a smaller amount remains in the dried technical powder material.

The water quality parameters were within the normally required ranges for daphnid testing (temperature ranged between 20 and 22 °C, pH between 7.7 and 9.6, dissolved oxygen ranged between 7.3 and 9.3, hardness at 180 mg/L and conductivity at 500 µmhos/cm (=µS/cm)). The slightly elevated conductivity levels are not considered to be of influence as they are still in the normal range of freshwater. The condition under which the test was performed are acceptable.

The toxicity of the tested fermentation recovery methods can be placed into context with the exposures and effect levels seen in the studies evaluated above. In the acute study up to 42 mg technical/L were tested and an LC50 of 12 mg/L derived. In the reproduction study up to 74 mg technical material/L was tested and a NOEC of 0.5mg/L (reproduction) and 5 mg/L (survival) derived. In this study 70 mg technical powder/L for each sample (2 fermentation recovery methods, 2 fermenters) were tested. These exposures are comparable to the highest concentration in the reproduction study. The results seen for the fermentation batches are consistent with the toxicity observed in the other studies and no difference between the methods were observed. Therefore, the acute and reproductive study are considered to adequately cover the results seen in the present study.

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

Reference 8.2.2/05

Reference:	S.Y. Feng (1966) - Experimental bacterial **infections** in the oyster *Crassostrea virginica* Published in Journal of Invertebrate Pathology 8, 505-511
Guideline:	Not stated
GLP:	No
Material and methods:	*Crassostrea virginica* were collected from the Navesink and Shrewsbury Rivers in New Jersey. The Oysters were inoculated by intracardial injection with Mycobacterium smegmatis, A-3 (a Pseudomonas-like bacterium) and Bacillus thuringiensis. The University of California supplied the Bacillus thuringiensis stock culture. Oysters were kept in 25 x 25 cm Pyrex battery jar aquaria containing seawater. Constant aeration was maintained in each aquarium and the water was changed at least twice daily. Temperature was controlled at specific temperatures by using thermostatically controlled glass emersion heaters (±2°C) in a constant temperature room. The influence of a number of temperatures were evaluated during the study. The inocula were introduced into the oysters via the ventricular route in volumes of 0.2 mL per oyster: oysters injected with equal volumes of sterile sea water were used as controls. For the Bacillus thuringiensis inoculum oysters were injected with 2.5 x 10^7 cells (50:50 vegetative cells and spores). The bacterial populations in the oysters were sampled at various time intervals by sacrificing both control and experimental oysters and plating out samples of heart blood and whole oyster homogenate. Histological techniques were employed to reveal injected bacteria in situ. In the B thuringiensis study one control and two experimental oysters were sacrificed at the following intervals: 4-hours, 1, 2, 3, 6 and 10 days.
Test substance	Bacillus thuringiensis, Mycobacterium smegmatis, Bacterium A-3 (Pseudomonas like organism)
Test species:	Crassostrea virginica
Number of test animals:	8 control and 16 treated
Treatments:	Injection of a Bt 2.5x 10^7 inoculum of vegetative cells and spores (50:50)
Duration:	Samples at different intervals (4 hrs to 12 days)

Test conditions:	The influence of a number of temperatures were evaluated during the study.
Endpoint:	All three species of bacteria used in the study, when injected into oysters, were eventually rendered non-viable by the host. *M. smegmatis* were rapidly destroyed in the host after day-5; A-3 ran a relapsing course of infection which was affected by ambient water temperature. B thuringiensis was rapidly cleared from the blood stream of the oyster. The B thuringiensis toxin did not affect oysters. Histological studies showed that in oysters injected with B thuringiensis and *M. smegmatis* most of the vegetative cells were quickly ingested by the leucocytes in the circulatory system. Migration of microorganism-laden leucocytes through the intestinal epithelium was seen occasionally. Occlusion of visceral mass blood vessels by leucocytes began 4-hours post injection with B thuringiensis and remained for at least 2-days before it was resolved. A similar host response was not seen with oysters injected with *M. smegmatis.*
Observations:	The B thuringiensis toxin did not affect oysters. Histological studies showed that in oysters injected with B thuringiensis and *M. smegmatis* most of the vegetative cells were quickly ingested by the leucocytes in the circulatory system

Results:

A summary of endpoints is given in the table below.

Table B.9.2.6.a: Toxicity effects / Infectivity / Pathogenicity of the MPCA to freshwater invertebrates

Test species	*Crassostrea virginica*
Toxicity	The *B thuringiensis* spores and vegetative cells at 2.5 x 10^7 / oyster (injected via 0.2 mL solution) and any toxins did not affect oysters. Histological studies showed that in oysters injected with *B thuringiensis* and *M. smegmatis* most of the vegetative cells were quickly ingested by the leucocytes in the circulatory system.
Infectivity / Pathogenicity	No signs of pathogenicity or infectivity observed.

Comments and conclusion RMS:

The study was previously evaluated in the DAR of 2007 and it was noted that the used Bt subspecies and strain were not reported.

The *B thuringiensis* spores and vegetative cells at 2.5 x 10^7 / oyster (injected via 0.2 mL solution) and any toxins did not affect oysters. Histological studies showed that in oysters injected with *B thuringiensis* and *M. smegmatis* most of the vegetative cells were quickly ingested by the leucocytes in the circulatory system.

The RMS maintains the conclusion from the previous evaluation and considers this study suitable as supporting information.

The notifier has submitted further information:

'Considerations to review the guidance for aquatic studies for microbial products

It has long been considered by industry that the current 21-day guideline for the conduct of daphnia studies is not appropriate for microbial pesticides. The question of how to conduct aquatic studies on Daphnia and algae has been now been raised at the level of OECD BPSG and the VGM for ecotoxicology testing. Daphnia do not thrive under turbid conditions. Due to the turbidity caused by the particulate nature of the microbial formulations along with the constant agitation required by exposure requirements, Daphnia actual algae feeding is reduced. It has been observed that Daphnia's feeding efficiency is reduced if in the presence of suspended clay particles, eventually starving if they are not removed from these turbid conditions. It has been shown from microcosm studies on Bacillus thuringiensis subsp. israelensis that no toxic effect is seen on Daphnia at the same concentrations as those used in laboratory studies. Bacillus thuringiensis subsp. aizawai strain ABTS-1857 does not have a direct toxic effect on Daphnia. In nature in such a case where water contamination with XenTari® WG were to occur, the particulate matter would rapidly sediment to the bottom of the pond or waterway, thus alleviating any possible effects on daphnia. The end points created by the current guidance are considered to be extreme and not reflective of true toxicity or what would occur in the actual environment.

Daphnia:

• Since daphnids seem to be non-selective filter feeders (i.e., they do not discriminate between individual food particles by taste) high concentrations of suspended material can interfere with the uptake of food particles. Although low concentration of suspended particles can actually be beneficial, as indicated by Rellstab and Stakk (2007), they also found decreases in Daphnia health at suspended particle concentrations of > 25 mg/L. Increases in suspended sediment concentrations (to 50 – 100 mg/L) have been shown to decrease ingestion rates to potential starvation levels (Arruda, Marzolf and Faulk, 1983).

• Laboratory experiments have shown that natural concentrations and particle sizes of suspended sediments reduce the fecundity, survivorship and fitness of cladocerans (Arruda et al., 1983; Kirk,

1991a; Kirk, 1991b; Kirk, 1992; Kirk & Gilbert, 1990; McCabe & O'Brien, 1983; Zurek, 1982), especially when simultaneously exposed to low algae concentrations.

• Most publications on Daphnia and suspended particles are short term studies. Robinson (2008) showed that exposure duration is more important than exposure concentration because for a range of concentrations the gut tract is filled with clay after some time being exposed and feeding is inhibited until they are placed in clean water. Just in differences between 12 and 24 hour exposure lead to:

1. increase in days to gravidity, compared to controls

2. significantly smaller than the controls

3. differences in LC50

Yet, regulatory guidance testing for microbial PPP require a 21 day- Daphnia test, not taking into account the physical parameters of the product.

While turbidity is something that should be taken into consideration as possibly causing adverse effects, consideration should be given to actual turbidity from the highest dose and the time between the subsequent applications. The product does not remain in suspension without agitation.

Different BTs are giving almost exactly the same NOAECs. Considering the specificity of BTs to target species, this raises the question as to whether toxicity is due to the microbe or some other parameter.

Other Bacillus:

Getting to Daphnia NOAECs on EPA's Fact Sheets, etc. is difficult for comparative purposes as some are done on spore numbers and others on mg/L. Not all fermentations will yield the same number of spores/g. Yet, it is evident that all bacteria are giving NOAECs in the same general concentrations.

B. pumilus QST 2808: The 21-day EC50 for daphnids was 6.2 x 10^5 CFU/mL in a 21-day static renewal bioassay test. Assuming direct application of QST 2808.

Bacillus subtilis QST 713: The 21-day EC50 for neonate cladocerans (Daphnia magna) is calculated as 1.6 x 10^6 CFU/mL and the NOAEC is 7.9 x 10^5 CFU/mL.

• Bacillus subtilis Strain QST 713 : The 21-day LC50 was determined to be greater than 3 x 10^7 CFU/L (1.5 mg/L). The lowest observable effect concentration (LOEC), based on reproduction, mean length and mean dry weight was determined to be 1.5 x 10^7 CFU/L (0.75 mg/L). The NOEC was determined to be 7.5 x 10^6 CFU/L (0.38 mg/L). The cause of death and whether pathogenicity was involved was not determined.

Bacillus licheniformis Strain SB3086 : The NOAEC was 1.2×10^6 CFU/mL based on survival and length measurements of the daphnids. The 21-day LC50 was 1.8×10^6 CFU/mL with 95% confidence limits of 1.6-2.0x10^6.

It is highly suspicious that all Bacillus are giving very similar endpoints. Once again, this raises the question as to whether toxicity is due to the microbe or some other parameter.

Overall Conclusion:

Daphnia study results with bacterial technical products are more a result of physical effects, than any true measure of bacterial toxicity, and therefore, the endpoints generated in studies according to the current guidance are not considered to be valid.'

Comments and conclusion RMS:

RMS agrees with the notifier, in the majority of cases the effects seen in the tests with daphnids are due to turbidity. RMS always discusses the physical effects during the study evaluation. Regarding the similar endpoints for different *Bacillus* strains, it is beyond the scope of this application to evaluate why the endpoints are in the same range.

Toxin/metabolite from microbial pest control agent (MPCA)

No study or information was submitted. According to the information provided in the former DAR: "*Bacillus thuringiensis* subsp. *aizawai,* Strain ABTS-1857, like other Bt strains commercially available, has been shown not to contain β-exotoxins or enterotoxins. Strict maintenance of environmental conditions and quality control analysis during the manufacturing process ensures the absence of potential microbial and non-microbial contaminants or potential animal or human pathogens".

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.

Furthermore, in Vol. 3 B6 it is stated that "*the insecticidal Cry toxins constitute 20-30% of the cell dry weight; synthesis of the toxins is controlled by a number of coordinating mechanisms at the transcriptional, post-transcriptional and post-translational levels. The pathogenicity of Bt to insects involves targeting specific cadherin receptors in the host; indicating that the mode of action involves effects on host-specific cell adhesion proteins*".

According to the information provided in the Vol.3 B8, "*although Bacillus thuringiensis subsp. azawai has been shown to survive to some extent in water, viability in the natural aquatic environment is influenced by many biological, chemical and physical factors. Predation by bacteriophages, protozoans and other lower animal forms undoubtedly plays a role in controlling the bacteriological population in the aquatic environment.*

Bacillus thuringiensis subsp. azawai is not regarded as an autochthonous inhabitant of aquatic environments and does not find optimal conditions for growth in the aquatic environment (e.g. waters are poor in organic carbon content). Therefore, proliferation is not likely to occur. Bacterial cells and espe-

cially spores may survive, but will be subject to natural competition in the diverse microbiota of natural waters. Survival of the applied ABTS-351 is not expected to cause any environmental or health impact".

Taking into account all the information, it is expected that the spores will just survive in the water, thus sporulation and germination will not occur, meaning no formation of metabolites.

B.9.2.7 Infectiveness to freshwater invertebrates

In total 5 studies were carried out with aquatic invertebrates of which four studies were performed with *Daphnia magna* (freshwater) and one study with the oyster, *Crassostrea virginica* (saltwater). In the four Daphnia studies infectivity was not investigated directly and purposefully, but at the same time no indications of such were reported in the studies. In the study with the oyster infectivity was well studied and not found to occur.

B.9.2.8 Pathogenicity to freshwater invertebrates

In total 5 studies were carried out with aquatic invertebrates of which four studies were performed with *Daphnia magna* (freshwater) and one study with the oyster, *Crassostrea virginica* (saltwater). In the four Daphnia studies pathogenicity was not investigated directly and purposefully, but at the same time no indications of such were reported in the studies. In the study with the oyster pathogenicity was studies and not found to occur.Effects on algae growth
During Annex I inclusion, data on effects on algae growth were submitted and evaluated only for the representative formulation. No data on the active substance are available. A formulation study was submitted. This study is summarized and evaluated in the product dossier.

B.9.2.9 Effects on plants other than algae

No new studies are submitted assessing the effect of BTa ABTS-1857 on plants other than algae (aquatic plants). Considering that the current mode of action concerns an insecticide, no studies with aquatic plants need to be submitted.

B.9.2.10 Summary of the studies on aquatic organisms toxicity, infectiveness and pathogenicity

Table 9.2.11: Summary of the studies on effects on aquatic organisms treated with the MPCA

Species	Test duration	Dose range	Results/ Endpoint	Observations	Reference

Species	Test duration	Dose range	Results/ Endpoint	Observations	Reference
TOXICITY					
Fish					
Rainbow trout *Oncorhynchus mykiss*	96 hours	100 mg/L test substance (2x107 CFU/mL	LC50: > 2.0 x 10^7 CFU/L	Infectivity was not investigated. Study duration too short for infectivity.	[redacted] 1991a 8.2.1/01
Rainbow trout *Oncorhynchus mykiss*	20 days	7.36 x 10^{10} CFU/L (aqueous) 1.47 x 10^8 CFU/g (dietary)	LC50 / NOEC: < 7.36 x 10^{10} CFU/L (nominal) < 5.4 x 10^{10} CFU/L (mean measured)	No signs of infectivity and pathogenicity. High turbidity levels may have lead to smaller and lighter fish in the treatments, which can have contributed to the effect.	[redacted] 1993 8.2.1/02
Rainbow trout *Oncorhynchus mykiss*	30 days	15, 60, 130, 250, 320, 390 and 460 mg test substance/L (aqueaous, nominal) 1.47 x 10^8 cfu/g	LC_{50} : 1.74 x 10^{10} CFU/L (mean measured)	Pathogenicity or infectivity were Not studied. Attenuated control (irradiation) lead to 100% mortality at day 4.	[redacted] 1995a 8.2.1/03
Invertebrates					
Waterflea *Daphnia magna*	10 day	1.9, 3.2, 5.4, 9.0, 15, 25 and 42 mg/L	LC_{50} (survival): 12 mg/L (2.4 x 10^8	Infectivity and pathogenicity not tested.	Collins 1995b 8.2.2/01

Species	**Test duration**	**Dose range**	**Results/ Endpoint**	**Observations**	**Reference**
		(nominal)	CFU/L) (mean measured)		
Waterflea *Daphnia magna*	21 days	0.5, 5 and 74 mg/L (nominal) 1.43 x 10^7, 1.94 x 10^8, 4.0 x 10^9 CFU/L (mean measured)	21-day NOEC (survival): 10^9 CFU/L (nominal) and 1.94 x 10^8 CFU/L (mean measured) 21-day NOEC (reproduction): 10^8 CFU/L (nominal), 1.43 x 10^7 CFU/L (mean measured)	Infectivity and pathogenicity not tested.	Boeri 1991b 8.2.2./02
Waterflea *Daphnia magna*	10 days	600 mg/L spore/crystal preparation, technical supernatant, technical pellet, techical supernatant heated, 100 mg/L technical powder	The supernatant and the pellet contribute to the toxicity, while the onset of toxicity differed somewhat between the different components. The Technical Pellet had the fasted onset of toxicty (ET50 24h), whereas the spore/crystal fraction (ET50 270h) and the Heated Technical Supernatant (ET50 196h) had the longest.		Ward et al 1993 8.2.2/03
Waterflea *Daphnia magna*	7 days	70 mg/L of two different manufacturing processes	N/A	100% mortality occurred in both groups at day 6-8	Collins 1995c 8.2.2/04
Oyster *Crassostrea virginica*		2.5 x 10^7 spores and vegetative cells/ oyster	N/A	Histological studies showed that in oysters injected with *B thuringiensis*	Feng 1966 8.2.2./05

Species	Test duration	Dose range	Results/ Endpoint	Observations	Reference
				and *M. smegmatis* most of the vegetative cells were quickly ingested by the leucocytes in the circulatory system.	
INFECTIVENESS					
Fish					
Rainbow trout *Oncorhynchus mykiss*	20 days	7.36 x 10^{10} CFU/L (aqueous) 1.47 x 10^{8} CFU/g (dietary)	LC50 / NOEC: < 7.36 x 10^{10} CFU/L (nominal) < 5.4 x 10^{10} CFU/L (mean measured)	No signs of infectivity and pathogenicity. High turbidity levels may have lead to smaller and lighter fish in the treatments, which can have contributed to the effect.	[redacted] *1993 8.2.1/02*
Oyster *Crassostrea virginica*		2.5 x 10^{7} spores and vegetative cells/ oyster	N/A	Histological studies showed that in oysters injected with *B thuringiensis* and *M. smegmatis* most of the vegetative	*Feng 1966 8.2.2./05*

Species	Test duration	Dose range	Results/ Endpoint	Observations	Reference
				cells were quickly ingested by the leucocytes in the circulatory system.	
PATHO-GENICITY					
Fish					
Rainbow trout *Oncorhynchus mykiss*	20 days	7.36 x 10^{10} CFU/L (aqueous) 1.47 x 10^{8} CFU/g (dietary)	LC50 / NOEC: < 7.36 x 10^{10} CFU/L (nominal) < 5.4 x 10^{10} CFU/L (mean measured)	No signs of infectivity and pathogenicity. High turbidity levels may have lead to smaller and lighter fish in the treatments, which can have contributed to the effect.	[redacted] *1993 8.2.1/02*
Oyster *Crassostrea virginica*		2.5 x 10^{7} spores and vegetative cells/ oyster	N/A	Histological studies showed that in oysters injected with *B thuringiensis* and *M. smegmatis* most of the vegetative cells were quickly ingested by	*Feng 1966 8.2.2./05*

Species	Test duration	Dose range	Results/ Endpoint	Observations	Reference
				the leucocytes in the circulatory system.	

* Fotnotstext

B.9.3 Effects on Bees

B.9.3.1 Toxicity to bees

Microbial pest control agent (MPCA)

Reference 8.3/01

A study on toxicity and pathogenicity of the MPCA on adult honey bees (*Apis mellifera*) was submitted and evaluated during the EU review for the inclusion of this strain in Annex I. Full details of this study as per the respective EU DAR (B.9.3; see below) are provided below along with the RMS study comments.

Reference:	R.L. Kirkland (1991a) - The effect of Bacillus thuringiensis, ABG-6305 technical powder, on the honey bee, Apis mellifera L. Unpublished report, Report number: CAR 196-90
Guideline:	Pesticide Assessment Guidelines, FIFRA Subdivision M, Microbial Pest Control Agents 154A-23, EPA Office of Pesticide Programs, November 1988 (draft).
GLP:	Yes (40 CFR, Part 160)
Material and methods:	Test material: ABG-6305. Lot No 42-221-BD. Bacillus thuringiensis var. aizawai, strain ABTS-1857 (potency 28151 IU/mg, 2 x 10^{11} cfu/g). A dose-response oral (continuous feeding) test was conducted three times in which three replicates of at least 25 bees (Apis mellifera) per cage were each exposed to ABG-6305 at nominal test concentrations of 0, 1, 10, 100 and 1000 ppm. The bees were collected from the brood frames (worker nursery bees, less than 1 week old) of healthy colonies. The bees were placed directly into test cages, which were made of wire mesh (12.7 x 12.7 x 12.7 cm) and food supplied in glass vials containing 50% honey-water solution. The feeding vials contained either the honey-water solution (untreated con-

	trol group) or one of the test substance concentrations made up in the honey-water solution. The vials were weighed prior to being placed in each cage and the bees allowed to feed ad libitum for 24 hours. The vials were then removed and weighed again to determine the actual amount of food consumed. Replacement vials containing the same freshly prepared solutions were provided each day. Vials of untreated honey-water solution were also attached to another treatment group and used to determine the losses due to evaporation. The ingested doses per bee were calculated according to the amount of food consumed per bee (net amount of food taken divided by the number of bees per cage when the vials were replaced) and the test concentration, taking into account evaporative losses. The bees were held in the dark (except during assessments) at 21 to 28°C and with a relative humidity of 40 to 87%. The number of dead bees was assessed daily and any adverse effects noted. Net food consumption was recorded daily. The feeding and assessment procedures were repeated at 24-hour intervals until mortality in the untreated control group reached at least 20%, when the test was terminated. At this time, the total number of bees in each cage was determined. The test was repeated three times and the results presented for the total of nine replicates per treatment level.
Micro-organism	ABG-6305 Bacillus thuringiensis var aizawai, strain ABTS-1857. technical powder, Lot no. 42-221-BD, Spore count 2 x 10^{11} CFU/g
Test species:	Honey bee (Apis mellifera)
Number of test animals:	25 bees x 9 replicates per treatment
Treatments:	1000, 100, 10, 1, 0 ppm in honey water solution (1:1 v/v) and honey-water syrup evaporation control
Duration:	trial 1 (11 days) trial 2 (12 days) and trial 3 (9 days)
Test conditions:	21-28 °C and 40-87 % RH; continous darkness except during observations
Deviations from guideline	None
Endpoint:	Test duration in the three tests (time taken for control mortality to exceed 20%) was 11, 12 and 9 days, respectively. Calculations of the mean ingestion levels for ABG-6305 showed that 5.4 x 10^{-2}, 4.8 x 10^{-3}, 4.9 x 10^{-4} and 5.1 x 10^{-5} mg were ingested per bee per day at the 1000, 100, 10 and 1 ppm levels, respectively. There was no evidence of any effect on food consumption due to the treatments (e.g. repellency). Little or no effects of the treatments were evident during the first 4 to 5 days of exposure. However, the level of mortality increased as the duration of exposure increased so that at the termination of each test (days 9 to 12), the

	average (for all nine replicates) percentage corrected mortality at 1000, 100, 10 and 1 ppm was 96.2, 44.3, 17.6 and 0.01%, respectively (re-calculated from mortality data for individual replicates and tests). Table 1 shows the percentage mortality for the ABG-6305 and untreated control groups over the termination period for the three tests (days 9 to 12). This indicates that mortality tended to increase with the duration of the study (and age of bees). Thus, for test 2 (terminated on day 12) the average (for the three replicates) percentage corrected mortality at 1000, 100, 10 and 1 ppm was 100, 80.8, 33.7 and 17.3%, respectively.

Table 1

Honey bee mortality following exposure to ABG-6305 (Bacillus thuringiensis subsp. aizawai)

Rate (ppm)	Day	Dead/Total	Mortality (%)	Corrected mortality (%)
0	9	69 / 387	17.83	-
1		62 / 357	17.37	0.00
10		84 / 373	22.52	5.71
100		105 / 298	35.23	21.18
1000		347 / 375	92.53	90.91
0	10	31 / 216	14.35	-
1		54 / 199	27.14	14.93
10		61 / 230	26.52	14.21
100		60 / 194	30.93	19.36
1000		207 / 216	95.83	95.13
0	11	41 / 216	18.98	-
1		62 / 199	31.16	15.03
10		81 / 230	35.22	20.04
100		80 / 194	41.24	27.47
1000		214 / 216	99.07	98.85
0	12	24 / 116	20.69	-
1		33 / 96	34.38	17.26
10		54 / 114	47.37	33.64
100		89 / 105	84.76	80.78
1000		114 / 114	100.00	100.00

Observations:	The results of this study indicate that ABG-6305 (Bacillus thuringiensis subsp. aizawai) was chronically pathogenic/toxic to honey bees when fed continuously over a period of 9-12 days. The LC_{50} was calculated to be 326 ppm ABG-6305

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* (Honey bee)
Toxicity	9-12 day dietary LD50: 326 ppm (mg/L)
Infectivity / Pathogenicity	Infectivity and pathogenicity not studied.

Comments and conclusion RMS:

The study was previously evaluated in the DAR of 2007.

The OECD 237 (2013) guideline was applied to this test. The OPPTS 885.4380 (USEPA 1996) for testing of microbial pesticides is also applicable and was considered in the evaluation of the study as well.

The following changes and additions were made to the old summary from the DAR:

Material and Methods:

The bees were continuously fed the test substance. Each concentration was tested in triplicate.

Treatments:

1000, 100, 10, 1, 0 ppm in honey water solution (1:1 v/v) and honey-water syrup evaporation control

Duration:

trial 1 (d1 – d11) trial 2 (d1 – d12) and trial 3 (d1 – d9)

The data from the three trials was combined.

The test trials had to be terminated after 9-12 days due to increasing control mortality. The prescribed test duration according to OPPTS 885.4380 is 30 days. It is acknowledged that this test duration is too long for bees to survive. According to the Canadian guidance document and acceptable control mortality should be ≤ 10% and can be achieved in tests with duration of 12-14 day. Starting day 8 the mortality in the control increased to 11.89% and reached 20.69% at day 12. Considering the recommendations of the Canadian guidance document, the results starting day 8 are less reliable due to the high control mortality. Based on the corrected percent mortality until including day 7, the LD50 is > 1000 ppm while at day 8 the LD50 is 736 ppm.

The concentrations tested in ppm can be translated in mg technical powder/L honey water. Considering a spore count of 2 x 10^{11} CFU/g technical powder, the concentration in honey water at 1000 mg/L and 736 mg/L is equivalent to 2 x 10^{11} CFU/L and 1.47 x 10^{11} CFU/L, respectively. Considering the small difference in the endpoint and the mortality in the control at day 8, the LD50 can be set at > 2 x 10^{11} CFU/L for a test duration of 7 days.

The two highest concentrations tested of 100 mg/L and 1000 mg/L (i.e. 2 x 10^{10} CFU/L and 2 x 10^{11} CFU/L, respectively) are in the range of the concentrations in the spray liquid of 2.5 x 10^{10} CFU/L - 1 x 10^{11} CFU/L as per GAP.

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

Reference 8.3/02

New study submitted for the purpose of this evaluation.

Reference:	Gray J. (2016): BTC Aizawi (Strain ABTS-1857): Honey Bee (*Apis mellifera*) larval toxicity test, single exposure. Valent Biosciences Corporation Unpublished report, report number: XY58HS at EnvigoCRS Limited
Guideline:	OECD 237 (July 2013)
GLP:	Yes

Material and methods:

Micro-organism	*Bacillus thuringiensis aizawi* Strain ABTS-1857, XenTari® Technical powder, 99% Bta fermentation solids with 5.8 x10^{10} cfu/g, LOT 31679A0003
Test species:	*Apis mellifera* (Honey bee)
Number of test animals:	In total 252 larvae by 12 larvae per replicate, 3 replicates per treatment, 5 test item treatments, 1 toxic reference treatment, 1 control treatment (definitive test)
Treatments:	Definitive test: Nominal test item: 6.25, 12.5, 25, 50 and 100 µg a.s./larva Nominal toxic reference, dimethoate: 8.8 µg a.s./larva A range finder was carried out before the test. Further details and results are reported in the study report.
Duration:	72 hours (days 4-7 of the experiment)
Test conditions:	The bee larvae for the definitive test were optained from a local apiarist, were 4 days old at study initiation and stemmed from three different hives

assigned to the three replicates per treatment. On arrival at the test facility on Day 1 of the study, bee larvae, fed 20 µL of diet A , in individual grafting cells in 48-well plates were housed in a desiccator cabinet with a water saturated atmosphere in the dark at ca. 34ºC. Larvae were fed 20 µL diet B on Day 3 and the dose was administered in 30 µL Diet C on Day 4. Larvae were fed 40 µL and 50 µL Diet C on Days 5 and 6 respectively. On each feeding occasion both pre and post dose administration both the feed and the 48-well plates were placed on a warming plate and the larvae were fed under subdued light. The feed was administered using a sterile pipette and the food was added along the wall of each grafting cell, taking care not to touch or drown the larvae.

Temperature ranged from 33.5 – 35.9°C

Assessments of mortality were made 24, 48 and 72 hours after dosing (Days 5, 6 and 7 respectively). In addition, the presence of uneaten food was recorded on these days.

The concentrations of viable active substance in stock solutions were determined using a standard agar pour-plate method. One mL stock solution was mixed with 9 mL Phosphate Buffered Saline (PBS) and further diluted using PBS in ten-fold dilution steps to give a range of dilutions. One mL stock solution dilutions were placed into empty sterile Petri dishes in triplicate and agar pour plates prepared by adding molten Tryptone Soya Agar (TSA). The plates were allowed to set and then incubated at 30 – 35 °C for three days. Following incubation the numbers of colonies of the test organism present on the plates were recorded and the a.s. concentration in the stock solutions calculated.

Deviations from guideline: None.

Endpoint: LD50

Observations: No mortality was recorded in the water control. The validity criteria according the guideline were filfilled (control mortality ≤ 15% and toxic reference motality ≥ 50%). Mortality of 6, 11, 8, 8 and 25% was recorded at rates of 6.25, 12.5, 25, 50 and 100 µg a.s./larva respectively, 72 hours after dose administration. Reduced diet consumption was recorded after 24 hours for 1, 2, 0, 1 and 2 of the 36 larvae dosed at 6.25, 12.5, 25, 50 and 100 µg a.s./larva respectively of which 1, 0, 1, 0 and 2 had died at 48 hours. Reduced diet consumption was recorded after 48 hours for one larva at 25 µg a.s./larva but this was alive at 72 hours.

The 72 hour LD50 value for XenTari® TP to honey bee larvae was therefore

estimated to be >100 µg a.s./larva.
The study was considered valid as control mortality was acceptable (≤ 15%) and application of the toxic reference, technical dimethoate, at 8.8 µg a.i./larva resulted in > 50% corrected mortality.

Table 8.3/01-1: Effects on *A. mellifera* larvae exposed to the active ingredient *Bacillus thuringiensis* subspecies *aizwai* (Strain ABTS-1857) in a 72 h single exposure toxicity test

Nominal concentration (µg a.s./larva)	**% Mortality**			**% Mortality following reduced food consumption**
	24 h	**48 h**	**72 h**	**72 h**
Water control	0	0	0	0
6.25	3	6	6	2.8
12.5	6	11	11	0
25	3	6	8	0
50	3	3	8	0
100	14	19	25	5.6
Dimethoate	11	44	67	27.7

The toxicity of active ingredient *Bacillus thuringiensis* subspecies *aizwai* Strain ABTS-1857 (XenTari® TP) to honey bee larvae was determined in a 72 h single exposure laboratory study according to OECD 237. The test design comprised a water control and XenTari® TP applied at nominal concentrations of 6.25, 12.5, 25, 50 and 100 µg a.s./larva. The 72 h LD50 was estimated to be > 100 µg a.s./larva. The study was considered valid as control mortality was acceptable (≤ 15%) and application of the toxic reference, technical dimethoate, at 8.8 µg a.i./larva resulted in > 50% corrected mortality.

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* (Honey bee)
Toxicity	LD50 > 100 µg a.s./larva (5.8 x 10^6 CFU/larva)
Infectivity / Pathogenicity	Infectivity and pathogenicity not studied.

Comments and conclusion RMS:

The study was submitted for the purpose of renewal.

The OECD 237 (2013) guideline was applied to this test. The OPPTS 885.4380 (USEPA 1996) for testing of microbial pesticides is also applicable and was considered in the evaluation of the study as well.

The study is well designed and carried out entirely according to the guidelines principles and requirements. In addition, the verification of exposure was carried on the level of determining the amount of active substance (in cfu/mL through plating) in the stock solutions, which were used to prepare Diet C on day 4 for single dosing. No comparison to the nominal concentrations in the stock solutions was made. Therefore, the RMS has made this comparison:

Stock solution	**Nominal Concentration[1] (mg a.s./mL)**	**Nominal Concentration[2] CFU/mL**	**Measured Concentration[3] CFU/mL**	**% nominal**
A	33,33	1,93E+09	5,53E+09	286
B	16,67	9,67E+08	2,73E+09	282
C	8,33	4,83E+08	9,17E+08	190
D	4,17	2,42E+08	4,5E+08	186
E	2,08	1,21E+08	2,27E+08	188

[1] based on report dilution scheme, p12 (calculations were checked)

[2] based on 5.8 x 10^8 CFU/g (specification of a.s. given in report)

[3] refer to Appendix 3 of report (calculations were checked)

It appears that the concentrations achieved in the stock solutions used to prepare the treatment diet C are between 180 and 286% and therefore 2-3 times higher than intended. It must be assumed that afterwards a certain loss of CFU will occur during preparation of the diet again, however, this will probably not set off the amounts found in the stock solutions. At the same time an amount 2-3 times higher than intended is not much for a microorganism and in the realm of the expected variability. Therefore the intended exposure was confirmed.

The concentrations in the diet were in the range of the concentrations in the spray liquid which is considered acceptable.

The LD50 proposed by the applicant is based on the nominal concentration of >100 µg a.s./larva. This is the equivalent of the highest concentration tested of 1 x10^{11} CFU/L diet which is as well equivalent to the highest test concentration in the spray liquid according to the current GAP. This is considered acceptable.

The study duration (72h according to OECD 237) is generally considered too short to study the toxicity and pathogenicity of a microorganism (according to OPPTS 885.4380 normally 30 days would be required although this is considered an extremely long duration for honey bees under test conditions). This should be kept in mind for the risk assessment.

In the context of OECD 237, the study is considered reliable without restrictions. An uncertainty remains as for micro-organisms the exposure should be prolonged and in the case of the acute tests with adults, no effects were recorded during the up to day 8.

Toxin/metabolite from microbial pest control agent (MPCA)

No study or information was submitted. According to the information provided in the former DAR: "*Bacillus thuringiensis* subsp. *aizawai,* Strain ABTS-1857, like other Bt strains commercially available, has been shown not to contain β-exotoxins or enterotoxins. Strict maintenance of environmental conditions and quality control analysis during the manufacturing process ensures the absence of potential microbial and non-microbial contaminants or potential animal or human pathogens".

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. Therefore, the pathogenicity of Bt to insects involves targeting specific cadherin receptors in the host; indicating that the mode of action involves effects on host-specific cell adhesion proteins.

B.9.3.2 Infectiveness to bees

Two studies with bees were performed with the active substance: one honey bee adult study and one honey bee larva study. In both studies infectivity was not studied.

B.9.3.3 Pathogenicity to bees

Two studies with bees were performed with the active substance: one honey bee adult study and one honey bee larva study. In both studies pathogenicity was not studied.

B.9.3.4 Effects on arthropods other than bees

Studies on toxicity and pathogenicity of the MPCA on non-target arthropods other than bees were submitted and evaluated during the EU review for the inclusion of this strain in Annex I. Full details of these studies are provided as per respective EU DAR are provided below. The RMS comments are included under the previous studies' summary and evaluation.

B.9.3.5 Toxicity to arthropods other than bees

Microbial pest control agent (MPCA)

Reference 8.4/01

Reference:	R.D. Nelson (1991a): The effect of *Bacillus thuringiensis*, ABG-6305 technical powder, on the predatory mite *Metaseiulus occidentalis* (Nesbit) and their host prey the two spotted spider mite *Tetranychus urticae* (Koch). Unpublished report, study number, project ID 91.042
Guideline:	Pesticide Assessment Guidelines, FIFRA Subdivision M, Microbial Pest Control Agents 154A-23, US EPA Office of Pesticide Programs.
GLP:	Yes (40 CFR, Part 160)
Material and methods:	Test material: ABG-6305. Lot No 42-221-BD. *Bacillus thuringiensis* var. *aizawai*, strain ABTS-1857 (potency 28151 IU/mg, 2 x 10^{11} cfu/g). Three tests were carried out on the predatory mite *Metaseiulus occidentalis* and one of these also included its host prey, the two-spotted spider mite, *Tetranychus uticae* (Koch). *M. occidentalis* was chosen as it is an important spider mite predator in crops where *Bacillus thuringiensis* products are used for the control of lepidopterous pests and is recognised as an important non-target arthropod by the US EPA. It was obtained from a commercial insectary while *T. urticae* was obtained from in-house cultures. The study consists of three tests. Test I assayed for adult predator (*M. occidentalis*) mortality and the effect on fecundity from contact by direct application and exposure to residues of the test substance. Test II assayed for immature predator (*M. occidentalis*) mortality by direct contact and residual exposure. Test III as-

	sayed the potential for indirect predator mortality by assaying the effect of the test substance on spider mite prey (*T. uitcae*). In the first test, a bioassay was conducted to assess effects on mortality and fecundity after direct application to adults together with exposure to surface residues. Bean leaf discs were placed on moist cotton wool (lower side up) and 10 *T. urticae* of all developmental stages transferred to each leaf disc, followed one day later by 8 gravid female *M. occidentalis* per disc. There were 12 replicate leaf discs for each of four treatments, a water-treated control and ABG-6305 at concentrations of 0.24, 2.4 and 24 g/L (nominally 0.1, 1.0 and 10x the field concentration, respectively). After 24 hours acclimatisation*, M. occidentalis* numbers were reduced to 6 per disc and the treatments applied with an airbrush sprayer (so that the discs were just covered – no volume given). The leaf discs were held in a controlled environment of 22-22.5^{o}C, 40-80% relative humidity and a 16-hour photoperiod*. M. occidentalis* mortality and egg number were assessed 48 hours after application. In the second test, 12-15 *T. urticae* of all developmental stages were transferred to whole bean leaves placed on moist cotton wool and two days later three *M. occidentalis* eggs were added. Again there were 12 replicates for each of the 4 treatments used in the first test. Treatments were applied directly to the eggs on the leaves. 72 hours later, the hatching rate was assessed together with the number of live, dead and escaped larvae. Counts were repeated daily until no more eggs were hatching in the control. The test was continued until the predatory mites were adult in the control, when the numbers of males and females in each replicate were determined. In the third test, there were three separate test systems for *T. urticae*: gravid females, protonymphs and eggs. Gravid female mites and protonymphs were transferred to bean leaf discs placed on moist cotton wool and after 24 hours acclimatisation numbers were reduced to 3 per disc. In the case of the adult system, all eggs present were removed, while for the eggs the gravid females were removed and the number of eggs present recorded. Assessments of live, dead and escaped adult/ immature mites or hatched eggs for the three stages, respectively, were made 2, 4, 6 and 8 days after treatment. In addition, in the case of the adults counts were made of any new eggs deposited. Each replicate disc was treated as in the first test and in the case of the adults and protonymphs the discs were transferred to a similarly treated leaf on a moist gauze pad in a modified Munger cell.
Micro-organism	ABG-6305 *Bacillus thuringiensis* technical powder, (comprised of *Bacillus thuringiensis* var *aizawai*, strain ABTS-1857). Lot no. 42-221-BD, Spore count 2 x 10^{11} CFU/g
Test species:	*Metaseiulus occidentalis* and *Tetranychus uticae*

Number of test animals:	Test I : 8 mites x 12 replicates; test II: 3 mites x 12 rep.; test III: 3 indiv. x 3 repl.
Treatments:	0.24, 2.4 and 24 g/L, equal to 10, 1 and 0.1x field rates (4540, 45.4 and 4.54 g/190 L H2O)
Duration:	Test I: 120 hrs; test II: variable; test III: 2,4,6 and 8 days
Test conditions:	Photoperiod: 16:8 L:D; RH: 40-80%; Temperature: 22-25.5 °C
Deviations from guide-line	Deviations applied to the protocol are considered not to affect adversely the tests
Endpoint:	Results Test I: Adult female *M. occidentalis* showed statistically significantly reduced survival at all three ABG-6305 dose levels, compared to the control (ANOVA, p = 0.05) and this effect was concentration-related. There was a statistically significant reduction in the numbers of eggs laid per surviving female (ANOVA, p = 0.05) at 0.24 and 2.4 g/L but not at the 24 g/L, compared to the control. The numbers of eggs per female were similar (overall range of 1.8 to 3.4) and it is suggested that the higher number at 24 g/L (compared to the two lower levels) was due to the reduced number of surviving females. The results are presented in Table 1.

Table 1

Effects of ABG-6305 (*Bacillus thuringiensis* subsp. aizawai) on adult *Metaseiulus occidentalis* (Test I)

Test concentration (g ABG-6305/L)	Percentage survival*	No. eggs/surviving female*
Control	88.19 a	3.40 a
0.24	74.35 b	2.20 bc
2.4	67.32 c	1.80 c
24	56.08 d	2.88 ab

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

	Results Test II: There were no effects of the ABG-6305 treatments on the hatching of *M. occidentalis* eggs in the second test. Mean percentage hatch ranged between 81.50% in the control to 92.61% at 2.4 g/L and none of the treatment groups differed significantly from the control or between each other (ANOVA, p = 0.05). There was an effect of hatching over time, with an interaction effect indicating a faster rate at 2.4 g/L, but this was not rate-dependent and so unlikely to be treatment-related. There were no significant effects on the percentage of males and females at adulthood, although the ABG-6305 treatments all had a higher proportion of females compared to the control (71.4 to 76.9% compared with 63.6%). Results Test III: In the third test, adult female T. urticae showed statistically significantly reduced survival at 24 g/L, compared to the control (ANOVA, p = 0.05). However, most of the mortality was attributed to a physical effect i.e. the mites were stuck to the leaf surface by the spray. Protonymphs showed a significant reduction in survival at all ABG-6305 treatment concentrations (ANOVA, p = 0.05). Mortality at 0.24 and 2.4 g/L showed a similar increase compared to the control while at 24 g/L it increased to over 80% and was also significantly different from the two lower rates. There was also a significant effect on egg hatch at all ABG-6305 treatment concentrations (ANOVA, p = 0.05) but this was due to an effect on rate. There was no significant difference in the total amount of eggs that hatched in each treatment by day 8, when the overall mean (all treatments) for egg hatch was 99.23%. The results are presented in Table 2.

Table 2

Effects of ABG-6305 (*Bacillus thuringiensis* subsp. aizawai) on *Tetranychus urticae* (Test III)

Test concentration (g ABG-6305/L)	Mean percentage survival (adults)*	Mean percentage survival (protonymphs)*	Mean time-weighted average percent egg hatch*
Control	88.31 ab	94.50 a	50.90 a
0.24	79.90 b	75.02 b	48.38 b
2.4	93.13 a	73.06 b	48.04 b
24	30.48 c	17.35 c	46.97 b

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

Observations:	The results of this study indicate that ABG-6305 (Bacillus thuringiensis subsp. aizawai) had significant adverse effects on the two-spotted spider mite, Tetranychus urticae, at all test concentrations (0.24 to 24 g/L). The results also indicate that ABG-6305 had significant adverse effects on

	the adult predatory mite, Metaseiulus occidentalis, again at all concentrations tested. In both cases there was generally a dose-related response, with mortality approaching 50% or greater at the 24 g/L concentration. There were no significant effects on the hatching rate of exposed predatory mite eggs at the end of the test. However, a difference in hatching rate was observed between the treatments.

Results:

A summary of endpoints is given in the table below.

Table B.9.3.5.a: Toxicity effects/infectivity/pathogenicity of the MPCA to arthropods

Test species	*Metaseiulus occidentalis* (predatory mite, predator) *Tetranychus urticae* (spider mite, prey)
Toxicity	*Metaseiulus occidentalis:* ER50 > 24 g a.s./L (56% survival) equals > 6.8 x 10^8 IU/L and > 4.8 x 10^{12} CFU/L. No effects on the hatching rate and no effects on the percentage of males and females at adulthood. *Tetranychus urticae*: ER50 < 24 a.s./L however mortality attributed to physical effects. Protonymphs survival affected at all concentrations from 0.24 g a.s./L to 24 g a.s./L (i.e. 4.8 x 10^{10} CFU/L to 4.8 x 10^{12} CFU/L).
Infectivity / Pathogenicity	Not determinded.

Comments and conclusion RMS:

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

The most recent guideline applicable to this test is OPPTS 885.4340 (1996). As the test was performed in 1991, the predecessor of this guideline was used. This is considered acceptable, however a comparison to the current guideline was made. The concentrations tested are up to the 10 times the max field application rate as per current GAP.

The RMS has translated the determined effects into IU/L and CFU/L (see table above) and maintains the conclusion from the DAR (2007) and considers this study suitable for use in risk assessment.

Reference 8.4/02

Reference:	R.L. Kirkland (1991b) The effect of Bacillus thuringiensis, ABG-6305 technical powder, on the insect egg parasitoid (Trichogramma pretiosum Riley). California Agricultural Research Inc., Unpublished report No.: CAR 104L-91
Guideline:	Pesticide Assessment Guidelines, FIFRA Subdivision M, Microbial Pest Control Agents 154A-23, US EPA Office of Pesticide Programs.
GLP:	Yes (40 CFR, Part 160)
Material and methods:	Test material: ABG-6305. Lot No 42-221-BD. Bacillus thuringiensis var. aizawai, strain ABTS-1857 (potency 28151 IU/mg, 2 x 10^{11} cfu/g). Three tests were carried out on the insect egg parasitoid, Trichogramma pretiosum Riley (Hymenoptera: Trichogrammatidae), one of which is reported elsewhere. The first of the two tests reported here was an assay to assess any adverse effects on the adult parasitoid following contact and feeding on food and water treated with ABG-6305. The second test reported here was designed to assess the effects of ABG-6305 on the survival and development of immature parasitoids (larvae) by treatment of infected host eggs. In both tests, T. pretiosum were obtained from a US Department of Agriculture laboratory and a commercial insectary. They were reared on eggs of Heliothis zea (Boddie) as these were found to give more robust parasitoids than those from another source reared on Sitotroga cerealella (Oliver). Parasitised eggs were incubated at 23 to 29°C and 40 to 87% relative humidity (nearer 100% for the second test) under a 16-hour photoperiod until emergence. Newly emerged parasitoids were held for 24 hours until use. In both tests, there were four treatments, a water-treated control and aqueous solutions of ABG-6305 at concentrations of 0.24, 2.4 and 23.6 g/L (nominally 0.1, 1.0 and 10x the field concentration, respectively). In the first test, the test cages comprised plastic tubes (1.2 cm diam. x 8 cm) closed at both ends with corks. Dental cotton was fixed onto the inner surface of one of the corks and this was saturated with water or the test solutions. The same solution was also used to coat the inner surface of the tube (no dosing volume given). Following air drying, the inner wall of the test cage was streaked with honey or a mixture of honey plus ABG-6305. Ten adult T. pretiosum were released into each cage with five replicates per treatment. Adult mortality was assessed after 24, 48, 72, 96, 120 and 144 hours.

	In the second test, adult T. pretiosum females were transferred into vials (15 x 44 mm) lined with paper towel and with a streak of honey:water solution (1:1, v/v) on the inner wall. There were ten females per cage with five replicates per treatment and these were provided with paper strips coated with H. zea eggs in the ratio of 17-30 eggs per female, for 4 hours. After parasitisation, the host egg strips were sprayed with 2 mL of treatment solution and then air dried. The egg strips were incubated under the same conditions as before in petri dishes (50 x 9 mm). The numbers of parasitised eggs were counted along with the number and sex of the adult insects emerging from the eggs.
Micro-organism	ABG-6305 Bacillus thuringiensis technical powder (comprised of Bacillus thuringiensis var aizawai, strain ABTS-1857), Lot no. 42-221-BD, Spore count 2 x 10^{11} CFU/g
Test species:	Trichogramma pretiosum
Number of test animals:	Test I: 10 adults x 5 replicates/3 concentrations; test II: 10 females x 5 replicates/3 concentrations
Treatments:	0.1, 1 and 10 field rate (0.24, 2.4 and 23.6 g a.s./L spray solution)
Duration:	Test I: 24, 48, 72, 96, 120, 144 hrs; test II: 10 days
Test conditions:	23-29 °C; 40-87% RH; photoperiod 16:8 L:D
Deviations from guideline	Deviations applied to the protocol are considered not to affect adversely the tests
Endpoint:	Test I: ABG-6305 had no significant adverse effects on the mortality of adult T. pretiosum when exposed by contact with fresh, dried residues and through ingestion at concentrations of up to 23.6 g/L. Test II: Similarly, there were no adverse effects on the number and sex ratio of adult T. pretiosum emerging from host eggs treated with ABG-6305 (initial parasitism levels were similar between the control and treated groups). The results are presented in Table 1.

Table 1

Effects of ABG-6305 (Bacillus thuringiensis subsp. aizawai) on adult Trichogramma pretiosum and parasitised Heliothis zea eggs

Test concentration (g ABG-6305/L)	Cumulative percent adult mortality (Day 6)*	Cumulative emergence (Day 10)*	Ratio Female / Male
Control	49.9 a	190.9 a	1.97
0.24	54.3 a	203.3 a	1.67
2.4	40.9 a	175.6 a	1.54
23.6	49.9 a	198.5 a	1.99

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

Observations:	The results of this study indicate that ABG-6305 (Bacillus thuringiensis subsp. aizawai) had no significant adverse effects on the adult parasitoid, Trichogramma pretiosum, when exposed by contact with fresh, dried residues and by ingestion at concentrations of 0.24 to 23.6 g/L. Similarly, there were no adverse effects on the number and sex ratio of adult T. pretiosum emerging from host eggs treated at the same concentrations.

Results:

A summary of endpoints is given in the table below.

Table B.9.3.5.a: Toxicity effects/infectivity/pathogenicity of the MPCA to arthropods

Test species	*Trichogramma pretinosum*
Toxicity	ER50 mortality: > 23.6 g a.s./L, equals > 6.6 x 10^8 IU/L and > 4.8 x 10^{12} CFU/L spray solution No effects on egg hatching and sex ratio.
Infectivity / Pathogenicity	Not determined.

Comments and conclusion RMS:

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

The most recent guideline applicable to this test is OPPTS 885.4340 (1996). As the test was performed in 1991, the predecessor of this guideline was used. This is considered acceptable, however a comparison to the current guideline was made.

The control mortality rose above 20% already on day 2 and reached 49.9% at day 6. According to the guideline "*control and treated insects should be observed for 21 to 30 days after dosing, if this is possible. In cases where the insect species cannot be cultured for 21 to 30 days, observation should continue until control mortality rises above 20 percent*". The life cycle of *Trichogramma* is about 8-10 days and that would explain why the control mortality increased to 50% at day 6. As the study is old and no indications for an effect of the active substance are seen, the result is considered acceptable. The concentrations tested were up to 48 times the max field application rate as per current GAP.

The RMS has translated the determined effects into IU/L and CFU/L (see table above) and maintains the conclusion from the DAR (2007) and considers this study suitable for use in risk assessment.

Reference 8.4/03

Reference:	A. Castellon (1991) The effect of Bacillus thuringiensis, ABG-6305 technical powder, on the insect egg parasitoid Trichogramma pretiosum Riley – **supplemental report in reference 8.4/02**. California Agricultural Research Inc., Unpublished report No.: CAR-104L-91
Guideline:	Pesticide Assessment Guidelines, FIFRA Subdivision M, Microbial Pest Control Agents 154A-23, US EPA Office of Pesticide Programs.
GLP:	Yes (40 CFR, Part 160)
Material and methods:	Test material: ABG-6305. Lot No 42-221-BD. Bacillus thuringiensis var. aizawai, strain ABTS-1857 (potency 28151 IU/mg, 2 x 10^{11} cfu/g). Following on from the previous study with the insect egg parasitoid, Trichogramma pretiosum Riley (Hymenoptera: Trichogrammatidae), a third test was conducted. This had to be repeated several times due to high control mortality. A number of improvements were introduced to achieve acceptable results: T. pretiosum was reared on Heliothis zea eggs (as these were found to give more robust parasitoids than those reared on Sitotroga cerealella Oliver); the number of assessments (experimental handling) was reduced i.e. no exposure of host eggs to the adult wasps at 4 days post-treatment. T. pretiosum was obtained from a US Department of Agriculture laboratory. They were reared on eggs of Heliothis zea (Boddie). Parasitised eggs were incubated at 23 to 29°C and 40 to 87% relative humidity under a 16-hour photoperiod until emergence. Newly emerged parasitoids were held for 24 hours until use. There were four treatments, a water-treated control and aqueous solutions of ABG-6305 at concentrations of 0.24, 2.4 and 23.6 g/L (nominally 0.1, 1.0 and 10x the field concentration, respectively). The test cages comprised plastic tubes (1.2 cm diam. x 8 cm) closed at both

	ends with corks. Dental cotton was fixed onto the inner surface of one of the corks and this was saturated with water or the test solutions. The same solution was also used to coat the inner surface of the tube (no dosing volume given). Following air drying (ca. 2 hours), the inner wall of the test cage was streaked with honey or a mixture of honey plus ABG-6305. Ten adult female T. pretiosum were released into each cage with five replicates per treatment. The test cages were held under controlled conditions at 22 to 30^{o}C. After 24 hours, mortality was assessed and surviving females were transferred into clean cages with water and honey. At 2 days post-treatment, mortality was again assessed and the parasitoids were provided with paper strips coated with H. zea eggs (average number per strip was 434 eggs). After the eggs had been in the cages for 1.5 hours they were removed and incubated under the same conditions as before in petri dishes (50 x 9 mm). Mortality counts were made daily for up to 6 days in total and additional egg strips were exposed at 6 days post-treatment. The numbers of parasitised eggs were counted along with the number and sex of the adult insects emerging from the eggs.
Micro-organism	ABG-6305 Bacillus thuringiensis technical powder (comprised of Bacillus thuringiensis var aizawai, strain ABTS-1857), Lot no. 42-221-BD, Spore count 2 x 10^{11} CFU/g
Test species:	Trichogramma pretiosum
Number of test animals:	10 females x 5replicates/3 concentrations
Treatments:	0.1, 1 and 10 field rate (0.24, 2.4 and 23.6 g/L techn.powder)
Duration:	12 days
Test conditions:	23-29 °C; 40-87% RH; photoperiod 16:8 L:D
Deviations from guideline	Unable to check
Endpoint:	ABG-6305 had no marked adverse effects on the mortality of adult T. pretiosum when exposed by contact with fresh, dried residues and through ingestion at concentrations of up to 23.6 g/L. The mortality results are presented as the mean of five replicates and are adjusted for numbers escaped or stuck in honey (assumed to not be treatment-related) but no statistical evaluation is presented. However, there is some indication of a treatment-related increase in mortality, especially at test concentrations of 2.4 and 23.6 g/L. No statistical difference between any of the treatments (ANOVA, p = 0.05)

	was found in the level of emergence from host eggs parasitised by female wasps 2 days post-treatment. At 6 days post-treatment the number of females surviving was low (especially at the higher dose levels), resulting in high levels of variation in the parasitisation data. There was a concentration-related reduction in the level of emergence but this may have been due, in part at least, to the low female survival at the higher dose levels. There was no relationship between the sex ratio of emerging wasps and the test concentration. The results are presented in Table 1.

Table 1

Effects of ABG-6305 (Bacillus thuringiensis subsp. aizawai) on adult Trichogramma pretiosum

Test concentration (g ABG-6305/L)	Cumulative percent adult mortality (Day 6)	Cumulative percent emergence (Day 12)*	Cumulative percent emergence (Day 10)**	Ratio Female / Male
Control	82.94	74.94	65.5	1.35
0.24	81.62	75.62	66.6	1.14
2.4	90.64	85.78	20.0	1.50
23.6	93.32	69.50	0.0	1.49

* From host eggs parasitised by females treated 2 days previously

** From host eggs parasitised by females treated 6 days previously

Observations:	The results of this study indicate that ABG-6305 (Bacillus thuringiensis subsp. aizawai) had no significant effects on the survival and fecundity of the adult parasitoid, Trichogramma pretiosum, when exposed by contact with fresh, dried residues and by ingestion at concentrations of 0.24 to 23.6 g/L. However, there was some indication of an increase in mortality by 6 days post-treatment and this may have been reflected by a reduction in their parasitisation potential at this time although high variability makes a conclusive assessment difficult.

Results:

A summary of endpoints is given in the table below.

Table B.9.3.5.a: Toxicity effects/infectivity/pathogenicity of the MPCA to arthropods

Test species	*Trichogramma pretiosum*
Toxicity	Indicative, but no clear effects on mortality at

	concentrations between 0.24 to 23.6 g a.s./L (equivalent to 4.8 x 10^{10} – to 4.7 x 10^{12} CFU/L and 6.8 x 10^{6} to 6.6 x 10^{8} IU/L). Equally inconclusive effects on fecundity may have been a consequence of affected survival.
Infectivity / Pathogenicity	Not determined.

Comments and conclusion RMS:

The study was previously evaluated in the DAR of May 2007 and shortcomings for interpretation of the results due to the high variability in the data were noted.

The most recent guideline applicable to this test is OPPTS 885.4340 (1996). As the test was performed in 1991, the predecessor of this guideline was used. This is considered acceptable, however a comparison to the current guideline was made.

The control mortality rose above 20% already on day 1 after treatment (33.6%) and reached 82.49% at day 6. According to the guideline "*control and treated insects should be observed for 21 to 30 days after dosing, if this is possible. In cases where the insect species cannot be cultured for 21 to 30 days, observation should continue until control mortality rises above 20 percent*". The life cycle of *Trichogramma* is about 8-10 days. Control mortality higher than 20% was already observed from day 1. In contrast to the previous study, here an indicative increase in mortality is seen, which could be a clear effect masked by the high control mortality.

The concentrations tested were up to 48 times the max field application rate as per current GAP.

The RMS maintains the conclusion from the DAR (2007) and considers this study inconclusive and therefore not suitable for risk assessment.

Reference 8.4/04

Reference:	S.J. Palmer, J.B. Beavers (1993) Xentari technical powder (ABG-6305): a dietary pathogenicity and toxicity study with the ladybird beetle (*Hippodamia convergens*). Unpublished report, Report number 161-126A
Guideline:	Pesticide Assessment Guidelines, FIFRA Subdivision M, Microbial Pest Control Agents 154A-23, US EPA Office of Pesticide Programs, 1989.
GLP:	Yes (40 CFR, Part 160)
Material and methods:	Test material: ABG-6305. Lot No 42-221-BD. *Bacillus thuringiensis* var. ai-**zawai**, strain ABTS-1857, fermentation solids and solubles (6.2 x 10^{10} cfu/g).

	Ladybird beetles (Hippodamia convergens) were exposed to a geometric series of three concentrations of ABG-6305 administered in their diet: 1500, 3000 and 6000 ppm. The beetles were obtained from a commercial breeder and held in disposable one pint rolled paper containers (9 cm diameter x 9 cm), covered with a plastic petri dish through which was inserted a glass vial containing the appropriate diet. The diets were prepared by mixing an appropriate amount of the test material in water and then adding honey; a negative control consisted of pure honey and there was also an attenuated control (concentration equal to the highest test concentration). The test animals were allowed ad libitum access to this throughout the test period (fresh diets were added weekly). The beetles were held at 11.4 to 13.4°C for a 13-day holding period and then at 4 to 9°C prior to test initiation for ease of handling. Twenty-five beetles were placed in each test chamber, with two replicate chambers per treatment. During the test the beetles were held nominally at 29 to 32°C with an 8-hour light/16-hour dark photoperiod. Actual test conditions were: temperature in the range 23.7 to 31.9°C (average 27.5°C); humidity in the range 36 to 88% (average 69%). Observations of mortality and clinical signs of toxicity/abnormal behaviour were made twice in the first four hours after test initiation and then daily until mortality in the control exceeded 20%.
Micro-organism	ABG-6305 *Bacillus thuringiensis* technical powder, (comprised of *Bacillus thuringiensis* var *aizawai*, strain ABTS-1857). Lot no. 42-221-BD, specific activity 6.2 x 10^{10} cfu/g
Test species:	ladybird beetle (Hippodamia convergens).
Number of test animals:	25 beetles x 2 replicates x 3 concentrations
Treatments:	1500, 3000 and 6000 ppm
Duration:	27 days
Test conditions:	29-32 °C; relative humidity: not indicated; photoperiod 8:16 L:D
Deviations from guideline	Deviations were supposed not to affect the experiment

Endpoint:	Samples were collected from the 1500 and 6000 ABG-6305 ppm test diets and the negative control (honey only) prior to initiation of the test and after 7 days. Additional samples at all test concentrations were collected from each fresh diet at the time of renewal (days 7, 14 and 21). The nominal concentrations of 1500, 3000 and 6000 ppm correspond to spore counts of 1.17 x 10^8, 2.34 x 10^8 and 4.69 x 10^8 cfu/mL, respectively. The results of the weekly dose verification samples gave counts ranging from 1.23 x 10^7 to 2.47 x 10^7, 4.30 x 10^7 to 6.80 x 10^7 and from 1.08 x 10^8 to 1.43 x 10^8 cfu/mL for the three concentrations, respectively. The average recovery was 14.3, 23.7 and 26.1%, respectively. The results are presented in terms of the mean measured concentrations. The test was terminated on day 27 when negative control mortality exceeded 20%. Mortality in both controls and in the treatment groups tended to show a similar, steady increase throughout the test, reaching a level of 20 to 22% in all cases. One instance each of immobility and lethargy were noted in the control replicates over the 27-day exposure period, while two instances of immobility and four instances of lethargy were noted in the treatment groups. All surviving beetles were normal in appearance and behaviour at the end of the test. The results are presented in Table 1.

Table 1

Effects of ABG-6305 (*Bacillus thuringiensis* subsp. *aizawai*) on adult *Hippodamia convergens*

Test concentration (ppm ABG-6305)	Cumulative adult mortality (Day 27)	Cumulative sub-lethal effects (Day 27)*	Cumulative per-cent mortality (Day 27)	Mean per-cent mortality
Control	6/25	0; 0	24	22
	5/25	0; 0	20	
Attenuated control	4/25	0; 0	16	22
	7/25	0; 0	28	
1500	4/25	0; 0	16	20
	6/25	0; 0	24	
3000	6/25	0; 0	24	22
	5/25	0; 0	20	
6000	5/25	0; 0	20	22
	6/25	0; 0	24	

* Immobile; lethargic

Observations:	The LC_{50} value for the ladybird beetle, *Hippodamia convergens*, exposed to ABG-6305 (*Bacillus thuringiensis* subsp. *aizawai*) in the diet for 27 days was determined to be greater than 1560 ppm ABG-6304 (nominally 6000 ppm), the highest concentration tested. The no observed effect concentration (NOEC) was 1560 ppm ABG-6305. There was no apparent indication of toxicity or pathogenicity at any concentration tested.

Results:

A summary of endpoints is given in the table below.

Table B.9.3.5.a: Toxicity effects/infectivity/pathogenicity of the MPCA to arthropods

Test species	*Hippodamia convergens* (ladybird beetle)
Toxicity	Dietary ER50 mortality: > 6000 ppm (> 4.69×10^{11} CFU/L diet, nominal measured);
Infectivity / Pathogenicity	No signs of pathogencity. Infectivity not tested.

Comments and conclusion RMS:

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

The most recent guideline applicable to this test is OPPTS 885.4340 (1996). As the test was performed in 1991, the predecessor of this guideline was used. This is considered acceptable, however a comparison to the current guideline was made. The concentrations tested are up to 4 times the max field application rate as per current GAP.

The RMS maintains the conclusion from the DAR (2007) and considers this study suitable for use in risk assessment.

Reference 8.4/05

Reference:	R.D. Nelson (1991b) The effect of *Bacillus thuringiensis*, ABG-6305 technical powder, on the common green lacewing *Chrysoperla carnea* (Stephens). Unpublished report, report number 91.043
Guideline:	Pesticide Assessment Guidelines, FIFRA Subdivision M, Microbial Pest Control Agents 154A-23, US EPA Office of Pesticide Programs, 1989.
GLP:	Yes (40 CFR, Part 160)
Material and methods:	Test material: ABG-6305. Lot No 42-221-BD. *Bacillus thuringiensis* var. *aizawai,* strain ABTS-1857, (2×10^{11} cfu/g). Three tests were carried out on the common green lacewing *Chrysoperla carnea*. *C. carnea* was chosen as it is an important spider mite predator in crops where Bacillus thuringiensis products are used for the control of lepidopteran pests and is recognised as an important non-target arthropod by the US EPA. The green lacewings were obtained from a commercial insectary. The study was spilt into three phases, although these all resulted from a single initial exposure to the test treatments: a water-treated control and ABG-6305 at concentrations of 0.24, 2.4 and 24 g/L (nominally 0.1, 1.0 and 10x the field concentration, respectively). In the first phase, 2-4 day old larvae of mixed sex were placed individually in cages (plastic cups with lids), together with lepidopteran eggs as food. There were four replicates of 25 larvae for each treatment. A single application of each treatment was made to the larvae, cage and food using an airbrush sprayer. All surfaces were applied to near run-off and left to dry before closing the cages with the treated lid. Additional treated eggs were provided on day 4. The larvae were assessed for mortality on days 2, 4 and 7 after application. The data from all three phases was statistically analysed

	using a 2-way ANOVA, with significantly different treatment means being separated using Duncan's Multiple Range Test. In the second phase, the larvae surviving from the initial exposure were evaluated for the number of days to pupation, total percentage pupation and the percentage emergence as adults. The larvae were fed untreated lepidopteran eggs at 2-day intervals from day 7 onwards. In the third phase, when the lacewing adults started to emerge they were sexed and placed in cages (Styrofoam cups placed in petri dishes) according to treatment. Food (wheat paste) and water-soaked cotton was also provided. Five pairs of adults were placed in each cage with four cages (replicates) per treatment. The average date of emergence was determined for each replicate and when the oviposition rate started to increase (usually 4-7 days after emergence) a new cage was provided and the number of eggs produced counted every 2-3 days. After each count a new cage was provided and this was continued for up to 14 days after emergence. The number of hatched eggs and the percentage egg hatch in each cage was assessed on a daily basis until no more eggs were seen to be hatching.
Micro-organism	ABG-6305 *Bacillus thuringiensis* technical powder, (comprised of *Bacillus thuringiensis* var *aizawai*, strain ABTS-1857). Lot no. 42-221-BD, Spore count 2 x 10^{11} cfu/g
Test species:	common green lacewing *Chrysoperla carnea*
Number of test animals:	Initially 25 larvae per replicate, 4 replicates per treatment.
Treatments:	0.24, 2.4 and 24 g a.s./L which corresponds to 0.1, 1.0 and 10 times field rate
Duration:	I phase: 2,4 and 7 days; II phase: not relevant; III phase: not relevant.
Test conditions:	19-33.5 °C; 26-68% RH
Deviations from guideline	The deviations reported do not reasonably affect the results of the experiment
Endpoint:	Larval mortality increased over the initial 7-day exposure period at the highest tested concentration of 24 g/L, reaching 61% on day 7, which was significantly greater than all the other treatments ($p = 0.05$). The mean time to pupation of the surviving larvae was significantly greater ($p = 0.05$) at 24 g/L (13.1 days) compared to the control (11.7 days). Similarly, the percentage of individuals reaching pupation was significantly reduced ($p = 0.05$) at 24 g/L (18.1% compared with 84% in the control) and also at 2.4 g/L (69% reaching pupation). The percentage of successful adult emergence from individuals that had pupated was not significantly different from the control in any of the

	treatments. However, the overall percentage of individuals reaching adulthood from larvae was significantly reduced compared to the control (p = 0.05) at 24 g/L, reflecting the effects at the earlier stages. The results are presented in Table 1

Table 1

Effects of ABG-6305 (Bacillus thuringiensis subsp. aizawai) on *Chrysoperla carnea* larvae

Test concentration (g ABG-6305/L)	Percentage mortality (Day 7)*	Mean number of days to pupation*	Percentage pupation*	Percentage adult emergence*	Percentage reaching adulthood*
Control	5 b	11.67 b	84 a	73.75 a	61 a
0.24	5 b	11.83 ab	80 ab	73.00 a	59 a
2.4	17 b	11.80 ab	69 b	74.25 a	52 a
23.6	61 a	31.10 a	18 c	78.75 a	14 b

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

	There was no significant difference in the number of eggs produced by adults from the control compared with the ABG-6305 treatments at 0.24 and 2.4 g/L. There was also no significant difference in the percentage egg hatch of the control compared with the 0.24 and 2.4 g/L treatments. In both cases, the 24 g/L could not be included in the statistical analysis as not enough adults for only one replicate was produced. However, there was no indication of any effects on the number of eggs laid or egg hatch at this level. The results are presented in Table 2.

Table 2

Effects of ABG-6305 (*Bacillus thuringiensis* subsp. *aizawai*) on *Chrysoperla carnea* egg production/hatch

Test concentration (g ABG-6305/L)	Mean number of eggs laid*	Mean percentage egg hatch*
Control	243.25 a	56.75 a
0.24	550.25 a	55.00 a
2.4	422.75 a	51.25 a
24	736.00**	66.00**

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

** Not a mean but a total from the single replicates (and so not included in statistical analysis)

Observations:	The results of this study indicate that ABG-6305 (Bacillus thuringiensis

	subsp. aizawai) had no significant effects on the survival and development time of *Chrysoperla carnea* larvae and also on the subsequent fecundity of the emerging adults at rates up to 2.4 g/L. The percentage of larvae reaching pupation however was significantly diminished at 2.4 g a.s./L. At 24 g/L, there were significant effects on larval mortality, larvae development time and pupation as well as emergence success, but not on the fecundity of any adults that subsequently emerged. However, the observations for fecundity in the highest dose originate from only one replicate and can therefore not be statistically validated.

Results:

A summary of endpoints is given in the table below.

Table B.9.3.5.a: Toxicity effects/infectivity/pathogenicity of the MPCA to arthropods

Test species	*Chrysoperla carnea* (green lacewing)
Toxicity	ER50 mortality: > 2.4 while < 24 g a.s./L (61% mortality at 24 g a.s./L), equivalent to > 6.8 x x10^7 while < 6.8 x 10^8 IU/L and > 4.8 x 10^{11} while < 4.8 x 10^{12} CFU/L, respectively ER50 reproduction (pupation success): > 2.4 g a.s./L equivalent to 67.6 IU/L and 4.8 x 10^{11} CFU/L
Infectivity / Pathogenicity	No signs of pathogenicity observed. Infectivity not assessed.

Comments and conclusion RMS:

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

The most recent guideline applicable to this test is OPPTS 885.4340 (1996). As the test was performed in 1991, the predecessor of this guideline was used. This is considered acceptable, however a comparison to the current guideline was made. The concentrations tested are up to 48 times the max field application rate as per current GAP.

No signs of pathogenicity were observed. Infectivity was not studied.

The RMS has translated the endpoints into IU and CFU units per L spray solution (see table above), maintains the conclusion from the DAR (2007) and considers this study suitable for use in risk assessment.

Toxin/metabolite from microbial pest control agent (MPCA)

No studies or information submitted.

No study or information was submitted. According to the information provided in the former DAR: "*Bacillus thuringiensis* subsp. *aizawai,* Strain ABTS-1857, like other Bt strains commercially available, has been shown not to contain β-exotoxins or enterotoxins. Strict maintenance of environmental conditions and quality control analysis during the manufacturing process ensures the absence of potential microbial and non-microbial contaminants or potential animal or human pathogens".

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. Therefore, the pathogenicity of Bt to insects involves targeting specific cadherin receptors in the host; indicating that the mode of action involves effects on host-specific cell adhesion proteins.

B.9.3.6 Infectiveness to arthropods other than bees

In total five studies were performed with various species of non-target arthropods other than bees. In none of these studies infectivity was studied.

B.9.3.7 Pathogenicity to arthropods other than bees

In total five studies were performed with various species of non-target arthropods other than bees. One study was considered sufficiently long to assess pathogenicity (Palmer 1993, Reference 8.4/03). Another study (Nelson 1991b) was comprised of different life stage related observation phases until adulthood after a single larval exposure. No signs of pathogenicity were observed in both studies.

B.9.3.8 Summary and risk assessment for non-target arthropod species other than bees

Species	Test duration	Dose range	Results/ Endpoint	Observations	Reference
TOXICITY					
Metaseiulus occidentalis	max 8 days	0.24, 2.4 and 24 g/L	LC50 mortality: > 24	Infectivity and pthogenciity	Nelson 1991a 8.4./01

Species	Test duration	Dose range	Results/ Endpoint	Observations	Reference
(predatory mite, predator) *Tetranychus urticae* (spider mite, prey)		(nominally 0.48, 4.8 and 48x the field concentration, respectively)	g a.s./L (56% survival, > 6.8 x 10^8 IU/L and > 4.8 x 10^{12} CFU/L) LC50 < 24 a.s./L however mortality attributed to physical effects. Protonymphs survival affected at all concentrations from 0.24 g a.s./L to 24 g a.s./L (i.e. 4.8 x 10^{10} CFU/L to 4.8 x 10^{12} CFU/L)	not tested.	
Trichogramma pretiosum	max 10 days	0.24, 2.4 and 23.6 g a.s./L	LC50 mortality: > 23.6 g a.s./L, equals > 6.6*10^8 IU/L and > 4.8 x 10^{12} CFU/L spray solution	Infectivity and pthogenciity not tested. High control mortality (49.9%).	Kirkland 1991b 8.4./02
Ladybird beetle *Hippodamia convergens*	27 days	1500, 3000 and 6000 ppm (conc tested are 1, 2 and 5 times field application rate)	Dietary LC50 mortality: > 6000 ppm (> 4.69x10^{11} CFU/mL diet, nominal, measured)	Infectivity not tested. No signs of pathogenicty.	Palmer 1993 8.4./04

Species	Test duration	Dose range	Results/ Endpoint	Observations	Reference
Green lacewing *Chysoperla carnea*	7 days	0.24, 2.4 and 24 g a.s./L	LC50 mortality: > 2.4 while< 24 g a.s./L (61% mortality at 24 g a.s./L), equivalent to **>** 6.8 x x10^7 while < 6.8 x 10^8 IU/L and > 4.8 x 10^{11} while < 4.8 x 10^{12} CFU/L, respectively LC50 reproduction (pupation success): > 2.4 g a.s./L equivalent to 67.6 IU/L and 4.8 x 10^{11} CFU/L	Infectivity not tested. No signs of pathogenicty.	*Nelson 1991b 8.4./05*
INFECTIVENESS					
None.					
PATHO-GENICITY					
Ladybird beetle *Hippodamia convergens*	27 days	1500, 3000 and 6000 ppm (conc tested are 1, 2 and 5 times field application rate)	Dietary ER50 mortality: > 6000 ppm (> 4.69x10^{11} CFU/mL diet, nominal, measured)	Infectivity not tested. No signs of pathogenicty.	Palmer 1993 8.4./04
Green lacewing *Chysoperla*	7 days	0.24, 2.4 and 24 g a.s./L	ER50 mortality: > 2.4	Infectivity not tested. No	*Nelson 1991b 8.4./05*

Species	Test duration	Dose range	Results/ Endpoint	Observations	Reference
carnea			while< 24 g a.s./L (61% mortality at 24 g a.s./L), equivalent to **>** 6.8 x x10^7 while < 6.8 x 10^8 IU/L and > 4.8 x 10^{11} while < 4.8 x 10^{12} CFU/L, respectively ER50 reproduction (pupation success): > 2.4 g a.s./L equivalent to 67.6 IU/L and 4.8 x 10^{11} CFU/L	signs of pathogenicty.	
Green lacewing *Chysoperla carnea*	7 days	0.24, 2.4 and 24 g a.s./L	ER50 mortality: > 2.4 while< 24 g a.s./L (61% mortality at 24 g a.s./L), equivalent to > 6.8 x x10^7 while < 6.8 x 10^8 IU/L and > 4.8 x 10^{11} while < 4.8 x 10^{12} CFU/L, respectively	Infectivity not tested. No signs of pathogenicty.	Nelson 1991b 8.4./05

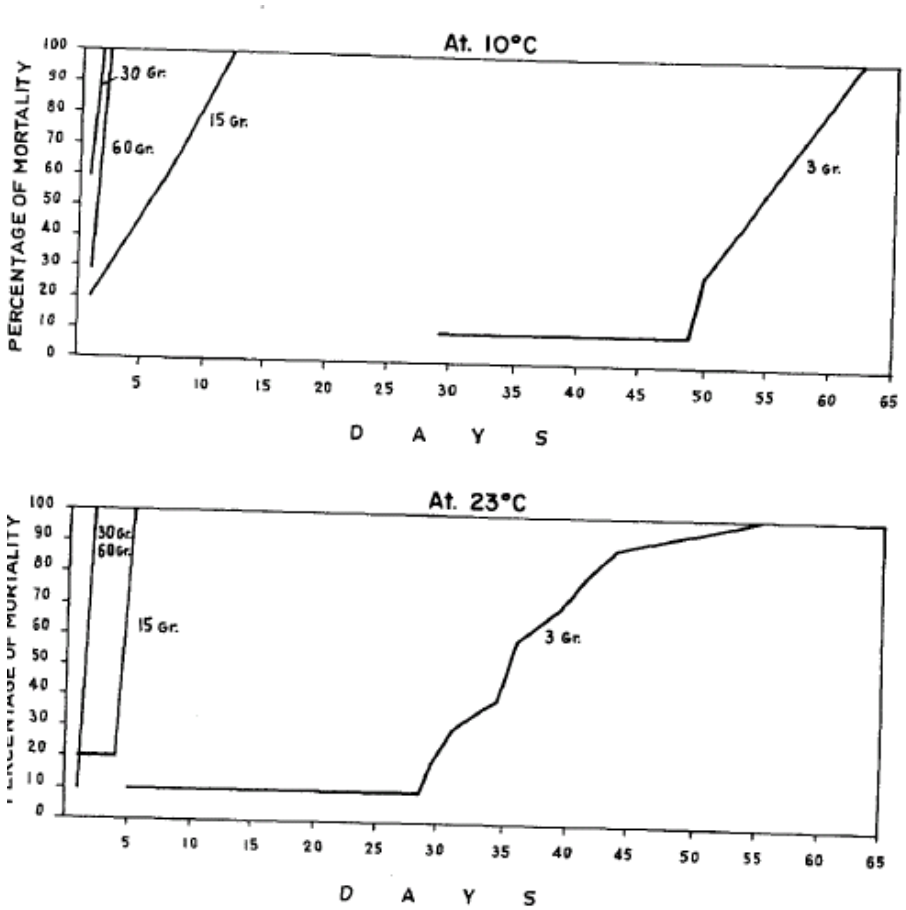
B.9.4 Effects on earthworms

B.9.4.1 Toxicity to earthworms

Microbial pest control agent (MPCA)

Reference 8.5/01

Reference:	Smirnoff, W.A. Heimpel, A.M. (1961) Notes on the **pathology** of *Bacillus thuringiensis* var. *thuringiensis* Berliner for the earthworm Published, Journal of insect Pathology 3, 403-408 (1961)
Guideline:	N/A
GLP:	No
Material and methods:	Test material: Bioferm "Thuricide" (50 x 10^9 spores/g). Preparation jars (100 mm diameter) were partially filled with 300 g of humus-rich, granular clay soil overlaid with rotting tree leaves (as food). Ten worms (approximately 4 inches long) collected from the same area were placed in each jar. After a short adaptation time, two bacterial preparations were mixed into the soil: test material at 30 x 10^9 spores/g and test material concentrate (50 x 10^9 spores/g). In both cases, separate jars were inoculated with 3, 15, 30 or 60 g of the bacterial preparation. Suitable control jars were also set up (no details given) and all experiments were repeated 3 or 4 times. The test units were separated into two equal lots, which were held at 10 or 23°C (90-95% relative humidity in both cases). Observations of mortality were made daily and dead worms were prepared for histopathological examination.
Micro-organism	*Bacillus thuringiensis* thuringiensis in Bioferm "Thuricide" (50 x 10^9 spores/g).
Test species:	Lumbricus terrestris
Number of test animals:	10 worms x 3-4 replicates x tested concentration
Treatments:	3, 15, 30 and 60 g/Thuricide per 300g soil (translated into 10, 50, 100 and 200 g product/kg soil and 5x10^{11}, 2.5x10^{12}, 5x10^{12},1x10^{13} CFU/kg soil).
Duration:	65 days
Test conditions:	10 or 23 °C and 90-95% RH
Deviations from guideline	-

Endpoint:	During a period of 4 months no worms died in the control jars. A dose dependent mortality was seen in all test treatments, with 100% mortality being reached after about 2 months with the lowest dose (3 g test material), while at the highest dose of 60 g test material this was reached in just over 10 days. There was some effect of temperature, with the time taken to reach 100% mortality decreasing at 23°C, particularly at the lower doses. There was also an effect of the concentration of the test material used, with the time taken to reach 100% mortality using the concentrate (50 x 10^9 spores/g) at 23°C being about 5 days or less with dose levels of 15 g and above. Preliminary histopathological examination showed that there was massive septicaemia in the infected worms before death. The bacteria were found to penetrate the fore part of the gut and invade all body tissues including the blood vessels. No bacteria were detected intracellularly but they were free to move between cells and could sporulate and form crystals in the body.  FIG. 2. Accumulative mortality of earthworms fed Bioferm "Thuricide" concentrate. (50 × 10^9 spores per gram.)
Observations:	On the basis of these results it was concluded that B. thuringiensis is pathogenic to earthworms. It was considered unlikely that earthworms would be seriously affected by living bacteria used in normal insecticidal applications.

	However, the ability of the spores to germinate in soil, penetrate the gut system of earthworms, move freely between cells, sporulate and form crystals in the body of the earthworm as well as the fate of the crystal endotoxin could be important for earthworm populations.

Results:

A summary of endpoints is given in the table below.

Table B.9.3.5.a: Toxicity effects/infectivity/pathogenicity of the MPCA to earthworms

Test species	*Lumbricus terrestris* (Earthworm)
Toxicity	50% mortality reached at 15g product/300g soil (50g product /kg soil and 2.5 x 10^{12} CFU/kg soil) within 6-7 days. 100% mortality reached at 3 g product/300 g soil within 2 month
Infectivity / Pathogenicity	Pathogenicity confirmed by the mortality and histopathological results seen. It was not clear, wheater the animals died through the toxin produced by Btt or by the microorgansim istself. Infectivity of cells was excluded, but infectivity of tissues and intracelluar spaces was confirmed.

Comments and conclusion RMS:

The study was previously evaluated in the DAR of May 2007 and considered acceptable. No guideline is applicable to this test as it is a research publication. The OECD guidelines 207 served as a rough orientation for the evaluation of this study.

The RMS determined the effects seen in the study in terms of a relevant effect seen within a relevant time frame. Within a timeframe of 10 days, the 50% mortality effects seen for 15g product/300 g soil were considered relevant. Within a long-term timeframe of 2 month also a 100% mortality was seen in the study at 3g product/300g soil. It is acknowledged that the Bt subspecies *thuringiensis* is not the current subspecies under consideration and the publication suffers from several shortcomings (, no presentation of raw data, statistics, lack of detailed description of the methodology, origin of the test substance), which affect the reproducibility and comparability of the study. According to Chapter 4.3.5 of OECD 67, "*futher analysis revealed that observed lethal effects were not attributed to mBCA. As explained in the review by Addison J.A. (1993) reported effects were caused by the presence diato-*

maceous earth used as carrier in the formulated product". Considering these the results cannot be used in the current risk assessment.

Reference 8.5/02

A strain-specific pathogenicity study for earthworms was not evaluated during the EU review for the inclusion of this strain in Annex I. A new study testing effects of BTa ABTS-1857 on earthworms has been conducted and is summarised below.

Reference:	A.B. Sindermann (2005) *Bacillus thuringiensis* subspecies *aizawai* technical powder: A pathogenicity study with the earthworm in an artificial soil substrate Unpublished report, Report number 529/1107005/Ew/Nca/SUB529
Guideline:	OECD Guideline 207 US EPA Series 885 – OPPTS Number 885.4340
GLP:	No
Material and methods:	Test material: *Bacillus thuringiensis* subspecies aizawai Technical Powder. Clitellate adult earthworms (Eisenia fetida) will be exposed to a single limit dose of 1000 mg test substance/kg dry soil in artificial soil. A control group, exposed to soil prepared without addition of the test substance will be maintained concurrently. In addition, reference substance groups will be exposed to chloracetamide at concentrations of 15 and 30 mg a.s./kg dry soil to verify the validity of the test system. The test substance will be administered in artificial soil (10% sphagnum peat, 20% kaolin clay and 70% industrial quartz sand plus calcium carbonate added as needed to adjust the soil pH to 6.0 ± 0.5) and water added to give a soil moisture content of 30 ± 5%. The treatment, control and reference substance groups will have four replicate test chambers (1 L all glass beakers), each containing 10 worms. To control bias, worms will be impartially assigned to exposure chambers at test initiation. The test will be conducted at 20 ± 5°C in continuous light. The worms will be provided air-dried, finely ground and pasteurised cow manure (1 g/100 g dry soil) as food, mixed into the test soil at the time of preparation. On day 14 following observations, approximately 5 g of dry manure, brought to the limit dose with test substance (0.1%), will be added to each replicate of the test substance group (5 g of untreated dry manure will be added to each control and reference substance replicate). Observations of mortality and other clinical signs will be made on days 7 and 14 and at test termination (day 30). The average weight of the live worms in each test chamber will be determined at the beginning and end of the test period. In addition, the burrowing

	behaviour of the worms will be observed on day 0 after they have been placed on the soil surface. The pH, temperature and moisture content of the artificial soil will be measured at each concentration at the beginning and end of the test.
Micro-organism	*Bacillus thuringiensis* subspecies *aizawai*
Test species:	*Eisenia fetida*
Number of test animals:	10 worms
Treatments:	1000 mg test substance/kg dry soil in artificial soil
Duration:	Observations were conducted at day 7, 14 and 30
Test conditions:	20 ± 5°C in continuous light Soil temperature 20 - 21 °C Soil moisture content 35.2 to 35.7 % at day 0. and 33.0 to 33.5 % at day 30. Soil pH 7 – 7.3 Photoperiod Continuous, with 569 ± 16.9 lux. Substrate: artificial soil according to OECD 207. Test soil was prepared by mixing B. thuringiensis subspecies aizwai, Strain ABTS-1857 Technical Powder with dry artificial soil then deionized water was added to achieve a moisture content of approximately 35% by weight. Air-dried, finely ground and pasteurised cow manure was added to the test soil at a rate of 1 g/100 grams dry soil. Test soil components were mixed for a total of approximately 25 minutes to ensure a homogeneous mix. Control and two reference groups (chloroacetamide, 15 and 30 mg a.i./kg dry soil) were also prepared in a similar manner. The test chambers were one litre glass beakers, covered with perforated plastic film to reduce evaporation.
Deviations from guideline	Some minor deviations
Endpoint:	Data from weekly observations showed no mortality in the control group and the Bt aizawai strain ABTS 1857 tech.powder group. In the 15 mg a.i. chloroacetamide/kg reference substance group, mortality was 27.5% (10 of 40) during the 30-day test. In the 30 mg a.i. chloroacetamide/kg referene substance group there was 100% mortality. Because there was 0 mortality in the test substance group, the LC_{50} was judged greater than the concentration tested. Earthworms in all groups were normal in appearance and behavior throughout the test period, except for two worms with reduced reaction to

<table>
<tr><td></td><td>mechanical stimulus in the 15 mg a.i. chloroacetamide/kg group on day 7. Earthworms in all groups exhibited no aversion to the soil during observations of burrowing behavior on days 0,7 and 14.</td></tr>
<tr><td>Observations:</td><td>On Day 0, group weighed worms were placed on the surface of the soil in each test chamber and observed twice for burrowing behaviour within the first hour. On days 7 and 14 contents of each test chamber were removed and the number of surviving worms were counted and observed for behavioural or pathological abnormalities. Following observations, test soil was returned to the test chambers, worms re-placed on the soil surface and observed for burrowing behaviour. On day 30 all surviving worms were removed from each replicate test chamber, rinsed with deionised water and blotted dry on paper towels. Group body weights were measured for each replicate, and average individual body weights were calculated.

No mortality occurred in the control or the 1000 mg a.i./kg soil dry weight test treatment. The toxic reference treatments resulted in 27.5 and 100 % mortality.
No sub-lethal effects (body weight) were seen during the study. No abnormal or avoidance behaviour was observed.
Bodyweights were similar in all groups with no evidence of treatment -related effects.

Table 8.5/01-1: Effects on E. foetida exposed to 1000 mg active ingredient Bacillus thuringiensis subspecies aizwai (Strain ABTS-1857)/kg soil dry weight in a 30-day toxicity test
<table>
<tr><td rowspan="3">Nominal active ingredient concentration
[mg a.i./kg soil]</td><td colspan="3">Mortality (initial population 40)</td><td colspan="2">Mean body weight</td></tr>
<tr><td colspan="3">Percentage</td><td colspan="2">Mean [g/worm]</td></tr>
<tr><td>7 d</td><td>14 d</td><td>30 d</td><td>0 d</td><td>30 d</td></tr>
<tr><td>Control</td><td>0</td><td>0</td><td>0</td><td>0.43</td><td>0.60</td></tr>
<tr><td>1000</td><td>0</td><td>0</td><td>0</td><td>0.43</td><td>0.60</td></tr>
</table>

The earthworm 30-day LC50 was estimated to be >1000 mg a.i./kg dry soil. The no observed effect concentration (NOEC) was found to be 1000 mg a.i./kg soil at day 30.</td></tr>
</table>

Results:

A summary of endpoints is given in the table below.

Table B.9.3.5.a: Toxicity effects/infectivity/pathogenicity of the MPCA to earthworms

Test species	Eisenia foetida
Toxicity	30-day LC50: >1000 mg a.i./kg dry soil (3.5×10^7 IU/kg dry soil) NOEC: 1000 mg a.i./kg dry soil
Infectivity / Pathogenicity	No signs of pathogenicity were observed. Infectivity was not studied.

Comments and conclusion RMS:

The study was newly submitted for the purpose of renewal.

The appropriate OECD and OPPTA guidelines have been used. The RMS has evaluated the study.

According to OECD 207 every single earthworm should be provided a single vial for the test and the 10 test animals per replicate are not supposed to be kept in the same beaker. In the present study 10 earthworms were kept in one 1L glass beaker filled with the artificial test soil. This seems to not have affected the control survival of the earthworms and is hence not considered an issue, which reduced the validity and reliability of the study.

The results were not expressed in CFU/kg dry soil. As the substance certificate only specifies IU (International Units) a conversion to CFU appears to be difficult.

In addition, the following results and conclusions are added.

Table 3
Average Body Weights of Earthworms Exposed to *Bacillus thuringiensis* subspecies *kurstaki*, Strain ABTS-351 Technical Powder in an Artificial Soil Substrate

Group	Replicate	Average Earthworm Body Weights (g)		
		Day 0	Day 30[1]	Change in Body Weight[2]
Control	A	0.40	0.52	+0.12
	B	0.45	0.66	+0.21
	C	0.44	0.60	+0.16
	D	0.41	0.62	+0.21
	Mean ± Std. Dev.	0.43 ± 0.024	0.60 ± 0.059	+0.18 ± 0.044
1000 mg *Bacillus thuringiensis* subspecies *kurstaki*, Strain ABTS-351 Technical Powder/kg dry soil (test substance group)	A	0.45	0.58	+0.13
	B	0.40	0.56	+0.16
	C	0.48	0.58	+0.10
	D	0.46	0.60	+0.14
	Mean ± Std. Dev.	0.45 ± 0.034	0.58 ± 0.016	+0.13 ± 0.025
15 mg a.i. chloroacetamide /kg dry soil (reference substance group)	A	0.41	0.42	+0.01
	B	0.42	0.56	+0.14
	C	0.45	0.52	+0.07
	D	0.44	0.61	+0.17
	Mean ± Std. Dev.	0.43 ± 0.018	0.53 ± 0.080	+0.10 ± 0.072
30 mg a.i. chloroacetamide /kg dry soil (reference substance group)	A	0.45	-	-
	B	0.40	-	-
	C	0.43	-	-
	D	0.44	-	-
	Mean ± Std. Dev.	0.43 ± 0.022	-	-

[1] Mean body weights were not statistically significantly different (p>0.05) for the 1000 mg *Bacillus thuringiensis* subspecies *kurstaki*/kg dry soil group and the 15 mg a.i/kg reference substance group when compared to the control group using Dunnett's 2-Talied Test of Means. All worms in the 30 mg a.i./kg reference group had died therefore Day 30 weights were not recorded.
[2] Change in body weight was not statistically significantly different (p>0.05) for the *Bacillus* group and for the 15 mg a.i./kg reference substance group when compared to the control group using Dunnett's 2-Tailed Test of Means.

There were no statistically different changes in body weight between the test substance group and the control.

The study is considered acceptable for use in risk assessment.

Toxin/metabolite from microbial pest control agent (MPCA)

No study or information was submitted. According to the information provided in the former DAR: "*Bacillus thuringiensis* subsp. *aizawai,* Strain ABTS-1857, like other Bt strains commercially available, has been shown not to contain β-exotoxins or enterotoxins. Strict maintenance of environmental conditions and quality control analysis during the manufacturing process ensures the absence of potential microbial and non-microbial contaminants or potential animal or human pathogens".

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. Therefore, the pathogenicity of Bt to insects involves targeting specific cadherin receptors in the host; indicating that the mode of action involves effects on host-specific cell adhesion proteins.

No further information was provided. Nevertheless, considering that the target organisms are leaf eating caterpillars belonging the order Lepidoptera, it is not expected that earthworms will be affected by

the cry proteins. As the study available indicates, there were no effects on earthworms after 30 days. Furthermore, the Bt is a soilborne microorganism thus evolutionary the earthworms have developed immune systems to cope with pathogens. This is highlighted in the OECD 67. Considering these, further information is not required.

B.9.4.2 Infectiveness to earthworms

Not addressed.

B.9.4.3 Pathogenicity to earthworms

No signs of pathogenicity were found at a dose of 1000 mg a.s./kg dry soil.

B.9.4.4 Summary for earthworms

Species	**Test duration**	**Dose range**	**Results/ Endpoint**	**Observations**	**Reference**
TOXICITY					
Earthworm *Eisenia foetida*	30 days	1000 mg test substance/kg dry soil in artificial soil	LD50: LD50: > 1000 mg a.s./kg dry soil	Not studied	*Sinderman 2005* 8.5/02
Earthworm *Eisenia foetida*	30 days	1000 mg a.s./kg soil dry weight	30-day LC50: >1000 mg a.i./kg dry soil (3.5 x 10^7 IU/kg dry soil) NOEC: 1000 mg a.i./kg dry soil	No signs of pathogenicity were observed. Infectivity was not studied.	*Sindermann 2006* 8.5/03
INFECTIVENESS					
Not investigated					
PATHO-GENICITY					
Earthworm *Eisenia foetida*	30 days	1000 mg a.s./kg soil dry weight	30-day LC50: >1000 mg a.i./kg dry soil (3.5 x 10^7 IU/kg dry soil)	No signs of pathogenicity were observed. Infectivity was	*Sindermann 2006* 8.5/03

Species	Test duration	Dose range	Results/ Endpoint	Observations	Reference
			NOEC: 1000 mg a.i./kg dry soil	not studied.	

B.9.5 Effects on non-target soil micro-organisms

No studies are available to assess the effects of 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 for the risk assessment, presented in product dossier.

B.9.6 Effects on terrestrial plants

Considering the mode of action no effects on terrestrial plants are expected.

B.9.7 Additional studies

No additional studies are available, not considered necessary.

B.9.8 References relied on

B.9.8.1 Literature search

Duffy L., 2016 Literature review report on *Bacillus thuringiensis aizawai* ABTS-1857 (Bta)

3. Summary: a brief summary indicating the purpose of the report, the methodology employed and the results obtained

This report summarises an evaluation of several literature searches performed according to to the EFSA document; Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009. EFSA Journal 2011; 9(2) : 2092 for public domain literature on *Bacillus thuringiensis aizawai* ABTS-1857 (Bta). Results of the search are provided below.

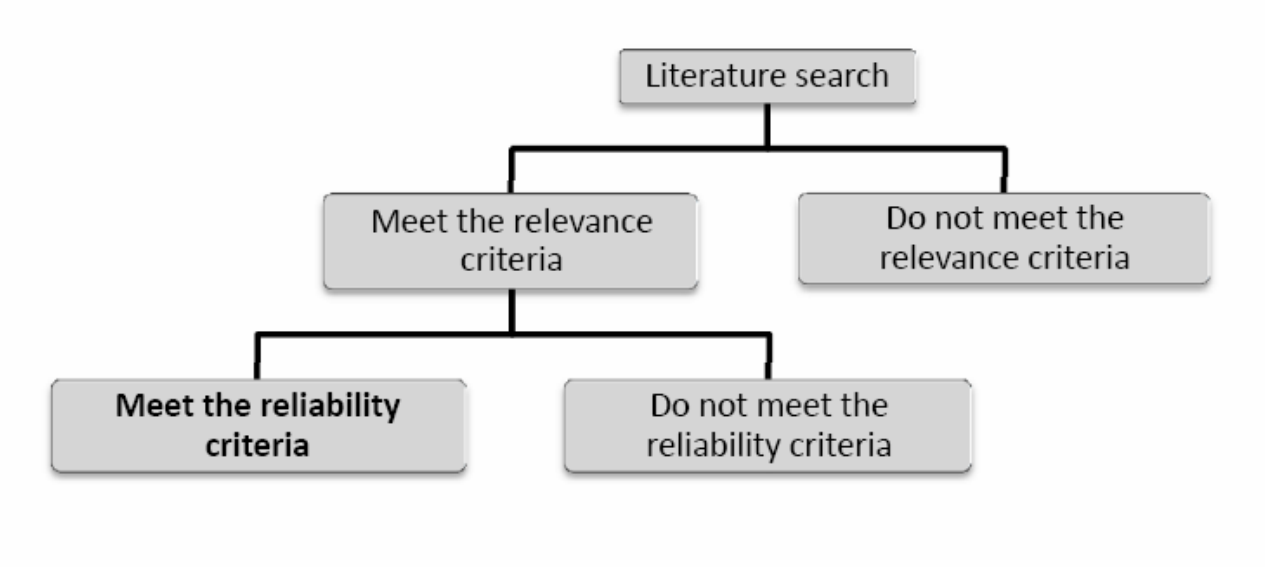
The search strategy was based on a single concept search. For details regarding the search strategy and the results obtained, please refer to section 5 of this Literature Review Report.

The selection process resulted in three categories of publication (as illustrated in the figure below):

Publications which meet the relevance criteria and are assessed to be reliable which are addressed
at the appropriate data points in the relevant section of the dossier.

Publications which meet the relevance criteria but are assessed to be non-reliable are referenced
and a justification for not meeting the reliability criteria provided in Section 6 of this Literature Review Report.

Publications not meeting the relevance criteria are referenced in Section 6 of this Literature Review
Report.



The relevance criteria applied are reported in section 4 (see Table 1) of this Literature Review Report. The reliability assessment for relevant studies was done according to Klimisch *et al*. (Klimisch, HJ, Andreae E, and Tillmann, U. 1997. A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data).

For identity and characterisation papers were deemed not reliable using the criteria:

- They were on genetically engineered strains or genetic constructs;
- Different methodology / manufacturing processes to the commercially relevant ABTS-1857 process;
- Strains that were not Bti, Bta or Btk;
- Conference posters, absence of the full paper in all libraries and databases in our reach (applying to some not in English);
- Paper contained only widely known background information that was described in the original submission.

The results of the review are provided in Tables 3, 4, 5 and 6 below.

Publications found to be relevant and reliable are addressed in the relevant section of the dossier and full-text documents are provided in the dossier. Publications found to be relevant and non-reliable are fully referenced and a justification for non-reliability is given in the overview table. Publications found to be non-relevant are fully referenced in the overview table.

4. Protocol containing the objective of the review and the criteria of relevance

4.1 Objective of the review

The review was made in order to identify scientific peer-reviewed open literature on the active substance *Bacillus thuringiensis aizawai* ABTS-1857 (Bta) which may affect the assessment on human health and/or the environment.

4.2 Criteria of relevance applied

Relevant publications are those showing new/unknown effects or information potentially contradictory to the regulatory data package for the active substance, on human health and/or the environment, which could impact the endpoints or the risk assessment parameters.

Studies providing information that supports the existing regulatory data package were considered as non-relevant.

The relevance criteria are presented in Table 1.

Table 1: Relevance criteria

Data requirement(s) indicated by the correspondent OECD data point number(s)	Criteria for relevance
Ecotoxicological studies (OECD code: IIA 8)	1. Well defined test material (including its purity and impurity profile). 2. Relevant test species 3. Number of animals per group sufficient to establish a statistical significance. 4. Several dose levels tested (at least 3), preferably including a negative control, to establish a dose-response. 5. Well described test methodology – appropriate guideline referenced 6. Description of the observations, mortality etc, and chemical analysis performed. 7. In addition: studies which may be helpful for the interpretation of other studies present in the dossier, but do not fit under a specific ecotoxicological endpoint.

5. Search methods and results

Details of the databases searched, justification for selection etc is provided below in Table 2.

No searches apart from bibliographic databases were undertaken.

Table 2: Reporting of the search process for scientific peer-reviewed open literature in bibliographic databases

Data requirement (s) captured in the search	**Details of the searches**				
Bacillus thuringiensis	**BIOSIS**	**Toxcenter**	**Medline**	**CAplus**	**CABA**
	Justification	Toxicology Cen-	MEDLINE is	Chemical Ab-	The CAB Ab-

aizawai ABTS-1857 (Bta) Covers all data requirements	**for choosing the source:** BIOSIS Previews® is the largest and most comprehensive life science database in the world. Amongst others subject coverage includes Agriculture, Biochemistry, Biophysics, Botany, Environmental Biology, Physiology, Toxicology. Sources include periodicals, journals, conference proceedings, reviews, reports, patents, and short communications. Nearly 6,000 life source journals, 1,500 international meetings as well as review articles, books, and monographs are reviewed for inclusion. Bibliographic information, indexing terms, abstracts, and CAS Registry Numbers are all searchable.	ter covers the pharmacological, biochemical, physiological, and toxicological effects of drugs and other chemicals. TOXCENTER is composed of the following subfiles: BIOSIS (1969 to date), CAplus (1907 to date), IPA (1970 to date), and MEDLINE (1953 to date). Sources include abstracts, books and book chapters, bulletins, conference proceedings, journal articles, letters, meetings, monographs, notes, papers, patents, presentations, research and project summaries, reviews, technical reports, theses, translations, unpublished material, web reprints. Records contain bibliographic data, abstracts, indexing terms, chemical names, and CAS Registry Numbers	the U.S. National Library of Medicine® (NLM) premier bibliographic database that contains more than 23 million references to journal articles in life sciences with a concentration on biomedicine. A distinctive feature of MEDLINE is that the records are indexed with NLM Medical Subject Headings (MeSH®). MEDLINE is the online counterpart to MEDLARS® (MEDical Literature Analysis and Retrieval System) that originated in 1964.	stracts Plus covers worldwide literature from all areas of chemistry, biochemistry, chemical engineering, and related sciences. Since October 1994 all articles from more than 1,600 key chemical journals are added including citations for documents not covered by CA. Coverage includes applied, macromolecular, organic, physical, inorganic, and analytical chemistry. Current sources include over 8,000 journals, patents and patent families from 38 national patent offices and 2 international patent organizations, technical reports, books, conference proceedings, dissertations, product reviews, bibliographic items, book reviews, and meeting abstracts. Electronic-only journals and Web preprints are also covered.	stracts database covers worldwide literature from all areas of agriculture and related sciences including biotechnology, forestry, and veterinary medicine. Sources for CABA include journals, books, reports, published theses, conference proceedings, and patents. Bibliographic information, indexing terms, abstracts, and CAS Registry Numbers are searchable.	

Date of the search: August 2016				
Date span of the search: 1 January 2005 – 23 August 2016 (BTa)				
Date of the latest database update included in the search: At 02/2016 24.8 mil-	**Date of the latest database update included in the search:** At 01/2016 12.2 mil-	**Date of the latest database update included in the search:** At 01/2016	**Date of the latest database update included in the search:** At 01/2016 8 million	**Date of the latest database update included in the search:** At 01/2016 8 million

lion records were available, the database is updated weekly.	lion records were available, the database is updated weekly.	25.3 million records were available, updated daily.	records were available, updated weekly	records were available, updated weekly

Search strategies used for this data requirement				
Terms searched:				
Ecotoxicology:				
non-target	fish or lepomis? or oncorhynchus or salmonidae or pimephales or cyprinid? or minnow or carp or zebraf? or goldfish? daphni? or asellus or chironom? or cloeon? or hyalella or 'aquatic invertebrate'		alga? or selenastrum? or scenedesmus? or chlorella? or skeletonema? or navicula? or anabaena?	
(nontarget? and plant) and (lemna? or chara? or elodea? or myriophyllum? or glyceria? or duckweed? or pondweed)			arthro? or lacewing? or hoverfly? or ladybird# or syrphid? or phytoseiulus? or aphidius? or typhlodromus?	ecotox?
adverse	pathogen?	enviro?	?toxi? and bird? or avian or mallard? or quail or bobwhite or repro? or oral or chronic	
honeybee# or bumblebee# or apis or bombus? or bee###		honeybee and contact or oral or larvae or feed?	earthworm? or eisenia? or lumbricus or allobophora? or dendrobaena? or aporrectodea? or dendrodrilus? or hypoaspis? or collembola? or springtail or terrestrial	
staphylinid? or coccinel? or pardosa? or orius? or bembidion? or hymenopt? or chrysopid?			bird? or avian or mallard? or quail or bobwhite or repro? or oral or chronic	

6. Results of the study selection process

In total, 519 summary records were retrieved from bibliographic databases and were screened by expert reviewers and grouped into two categories according to their likely relevance after rapid assessment of titles and, when available, abstracts:

1. Obviously not relevant: 485 summary records.

These summary records (titles and/or abstracts) did not contain specific information relevant to the criteria specified in Table 1.

2. Not excluded after rapid assessment: 34 summary records were classified as potentially relevant and thus were assessed in detail, a full assessment of the full-text documents.

3. Following assessment 26 of the full text documents were excluded from the dossier.

4. Following assessment 8 of the full text documents were included in the dossier.

The results are presented in detail below in Tables 3, 4, 5 and 6.

Table 3: Results of the study selection process, for each data requirement or group of data requirements searched

Data requirement(s) captured in the search (as indicated in Table 2)	**n**
Total number of *summary* records retrieved after *all* searches of peer-reviewed literature (excluding duplicates)	519
Number of *summary records* excluded from the search results after rapid assessment for relevance	485
Total number of *full-text documents* assessed in detail	34
Number of *studies* excluded from further consideration after detailed assessment for	26

relevance	
Number of *studies* included for relevance after detailed assessment	8

Table 4: Report of all relevant studies that are included in a dossier after detailed assessment of full-text documents for relevance: ordered by data requirement(s)

Data requirement (indicated by the corresponding OECD data point number)	Author(s)	Year	Title	Source
EFFECTS ON NON-TARGET ORGANISMS				
MMP 10.3.2	Mommaerts, Veerle; Jans, Kris; Smagghe, Guy [Reprint Author]	2010	Impact of *Bacillus thuringiensis* strains on survival, reproduction and foraging behaviour in bumblebees (Bombus terrestris).	Pest Management Science, (MAY 2010) Vol. 66, No. 5, pp. 520-525. ISSN: 1526-498X.

Table 5: Report of all relevant studies that are included in a dossier after detailed assessment of full-text documents for relevance: ordered by author(s)

Data requirement (indicated by the corresponding OECD data point number)	Author(s)	Year	Title	Source
EFFECTS ON NON-TARGET ORGANISMS				
MMP 10.3.2	Mommaerts, Veerle; Jans, Kris; Smagghe, Guy [Reprint Author]	2010	Impact of *Bacillus thuringiensis* strains on survival, reproduction and foraging behaviour in bumblebees (Bombus terrestris).	Pest Management Science, (MAY 2010) Vol. 66, No. 5, pp. 520-525. ISSN: 1526-498X.

Table 6: Report of studies excluded from the risk assessment after detailed assessment of full-text documents

Author(s)	Year	Title	Source	Reason(s) for not including this study in the dossier (reliability criteria, point 3 above)
EFFECTS ON NON-TARGET ORGANISMS				
Aggarwal, N.; Holaschke, M.; Basedow, T.	2006	Evaluation of bio-rational insecticides to control Helicoverpa armigera (Huebner) and Spodoptera exigua (Huebner) (Lepidoptera: Noctuidae) fed on Vicia faba L.	Mitteilungen der Deutschen Gesellschaft fuer allgemeine und angewandte Entomologie (2006), Volume 15, pp. 245-250, 16 refs. ISSN: 0344-9084 Published by: Deutsche Gesellschaft fuer allgemeine und angewandte Entomologie e.V., Bayreuth Conference: Papers from the Entomological Conference in Dresden, Germany, 21-24 March 2005.	Not determined to be relevant

Author(s)	Year	Title	Source	Reason(s) for not including this study in the dossier (reliability criteria, point 3 above)
Apaydin, Ozgur; Cinar, Celenk; Turanli, Ferit; Harsa, Sebnem; Gunes, Hatice [Reprint Author]	2008	Identification and bioactivity of native strains of *Bacillus thuringiensis* from grain-related habitats in Turkey.	Biological Control, (APR 2008) Vol. 45, No. 1, pp. 21-28. ISSN: 1049-9644.	This paper appears to relate to cereal grain which is not relevant for the uses proposed for this submission (brassicas, tomatoes and peppers). The paper would not therefore be considered by the authorities in support of this submission.
Basedow, T.; Ahmad, M.; Tadesse, B.; El-Shafie, H.	2008	Galleria mellonella (L.) (Pyralidae) and Spodoptera exigua (Huebner) (Noctuidae): differences in effects of XenTari® *(Bacillus thuringiensis aizawai*), NeemAzal T/S® and their combinations on survival.	Mitteilungen der Deutschen Gesellschaft fuer allgemeine und angewandte Entomologie (2008), Volume 16, pp. 365-368, 24 refs. ISSN: 0344-9084 Published by: Deutsche Gesellschaft fuer allgemeine und angewandte Entomologie e.V., MXFFFDncheberg Conference: Vortraege der Entomologentagung, Innsbruck, Austria, 26 February to 1 March, 2007. URL (Availability): http://www.dgaae.de	Not determined to be relevant
Basedow, T.; El-Shafie, H. A. F.; Abo-El-Saad, M. M.; Al-Ajlan, A. M.	2012	Evaluation of *Bacillus thuringiensis aizawai* and neem for controlling the larvae of the Greater Wax Moth, Galleria mellonella (Lepidoptera: Pyralidae).	International Journal of Agriculture and Biology (2012), Volume 14, Number 4, pp. 629-632, 21 refs. ISSN: 1560-8530 Published by: Friends Science Publishers, Faisalabad URL (Availability): http://www.fspublishers.org/ijab/past-issues/IJABVOL_14_NO_4/27.pdf	Not determined to be relevant
BenFarhat-Touzri, Dalel; Saadaoui, Marwa; Abdelkefi-Mesrati, Lobna; Saadaoui, Imen; Azzouz,	2013	Histopathological effects and determination of the putative receptor of *Bacillus thuringiensis* Cry1Da toxin in *Spodoptera littoralis* mid-	Journal of Invertebrate Pathology, (FEB 2013) Vol. 112, No. 2, pp. 142-145. http://www.journals.elsevier.com/journal-of-invertebrate-pathology/#description. CODEN: JIVPAZ. ISSN: 0022-2011. E-ISSN: 1096-0805.	Not determined to be relevant

Author(s)	Year	Title	Source	Reason(s) for not including this study in the dossier (reliability criteria, point 3 above)
Hichem; Tounsi, Slim [Reprint Author]		gut.		
El-Aziz, S. H. A.; El-Gohary, E. E.; Mansy, M. S.; Desuky, W. M.; Hamed, M. S.	2012	Toxicological and biochemical studies on development of resistance in *Spodoptera littoralis* (Boisd.) during selection with *Bacillus thuringiensis* MVPII.	The Journal of American Science (2012), Volume 8, Number 1, pp. 418-426, 39 refs. ISSN: 1545-1003 Published by: Marsland Press, New York URL (Availability): http://www.jofamericanscience.org/journals/am-sci/am0801/059_7571am0801_418_426.pdf	Not determined to be relevant
Gonzalez-Cabrera, J.; Molla, O.; Urbaneja, A.	2009	Biological control of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) with *Bacillus thuringiensis* (Berliner). Control biologico de *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) con *Bacillus thuringiensis* (Berliner).	Agricola Vergel: Fruticultura, Horticultura, Floricultura, Citricultura, Vid, Arroz (2009), Volume 28, Number 333, pp. 476-480, 17 refs. ISSN: 0211-2728 Published by: Ediciones y Promociones L.A.V., Valencia URL (Availability): http://www.edicioneslav.com	Not determined to be relevant
Hatem, A. E.	2014	Lethal and sublethal effects of spinosad and *Bacillus thuringiensis* aizawai on reproductivity of *Spodoptera littroralis* (boisd.) and *Spodoptera exigua (*huebner) (Lepidoptera: Noctuidae).	Egyptian Journal of Biological Pest Control (2014), Volume 24, Number 1, pp. 65-69, 22 refs. ISSN: 1110-1768 Published by: Egyptian Society for Biological Control of Pests, Cairo URL (Availability): http://www.esbcp.org/index.asp	Not determined to be relevant
Hommes, M.	2010	Investigations on integrated pest man-	Julius-Kuehn-Archiv (2010), Number 428, pp. 215-216 ISSN: 1868-9892 Published by: Julius Kuehn Institut, Bundes-	Not determined to be relevant

Author(s)	Year	Title	Source	Reason(s) for not including this study in the dossier (reliability criteria, point 3 above)
		agement in white cabbage. Untersuchungen zur integrierten Bekaempfung von Schaedlingen an Weisskohl.	forschungsinstitut fuer Kulturpflanzen, Quedlinburg Conference: 57. Deutsche Pflanzenschutztagung, Berlin, Germany, 6-9 September, 2010. URL (Availability): http://pub.jki.bund.de/index.php/JKA/issue/archive	
Kim, Min-Ju; Han, Jae-Kwang; Park, Jong-Su; Lee, Jin-Sung; Lee, Soon-Ho; Cho, Joon-Il; Kim, Keun-Sung [Reprint Author]	2015	Various Enterotoxin and Other Virulence Factor Genes Widespread Among Bacillus cereus and Bacillus thuringiensis Strains.	Journal of Microbiology and Biotechnology, (JUN 2015) Vol. 25, No. 6, pp. 872-879. http://jmb.or.kr. ISSN: 1017-7825. E-ISSN: 1738-8872.	Not determined to be relevant
Kumar, Prabhat [Reprint Author]; Huang, Lu-Ying Zoe; Srinivasan, R.	2014	Effect of three commercial biopesticides of neem (Azadirachta indica) and Bacillus thuringiensis on legume pod borer (*Maruca vitrata*) (Lepidoptera: Crambidae) in Thailand.	International Journal of Tropical Insect Science, (JUN 2014) Vol. 34, No. 2, pp. 80-87. http://journals.cambridge.org/action/displayJournal?jid=JTI. ISSN: 1742-7584. E-ISSN: 1742-7592.	Not determined to be relevant
Mashtoly, Tamer A.; Abolmaaty, Assem; El-Zemaity, Mohamed El-Said; Hussien, Mohamed I.; Alm, Steven R. [Reprint Author]	2011	Enhanced Toxicity of *Bacillus thuringiensis* Subspecies kurstaki and aizawai to Black Cutworm Larvae (Lepidoptera: Noctuidae) With *Bacillus* sp NFD2 and *Pseudomonas* sp FNFD1.	Journal of Economic Entomology, (FEB 2011) Vol. 104, No. 1, pp. 41-46. CODEN: JEENAI. ISSN: 0022-0493. E-ISSN: 1938-291X.	Not determined to be relevant
Maxwell, Elly M.	2006	Evaluation of several re-	Florida Entomologist, (JUN 2006) Vol. 89, No. 2, pp. 117-126. CODEN: FETMAC.	Not determined to be relevant

Author(s)	Year	Title	Source	Reason(s) for not including this study in the dossier (reliability criteria, point 3 above)
[Reprint Author]; Fadamiro, Henry Y.		duced-risk insecticides in combination with an action threshold for managing lepidopteran pests of cole crops in Alabama.	ISSN: 0015-4040.	
Mommaerts, V.; Sterk, G.; Smagghe, G. Editor(s): Oomen, P. A.; Thompson, H. M.	2009	Side effects of commercial *Bacillus thuringiensis* insecticides on micro-colonies of *Bombus terrestris.*	Julius-Kuehn-Archiv (2009), Number 423, pp. 68-69 ISSN: 1868-9892 Published by: Julius Kuehn Institut, Bundesforschungsinstitut fuer Kulturpflanzen, Quedlinburg Conference: Hazards of pesticides to bees. 10th International Symposium of the ICP-Bee Protection Group. Bucharest, Romania, 8-10 October, 2008. URL (Availability): http://pub.jki.bund.de/index.php/JKA/issue/archive	Conference abstract only. Full study details are available in the published paper referenced also in the literature search.
Pandey, Shachindra [Reprint Author]; Joshi, Bishwambhar D.; Tiwari, Lakshmi D.	2009	Relative efficacy of two subspecies of *Bacillus thuringiensis*, available as commercial preparations in market, on different stages of a lepidopteran pest, *Spodoptera litura* (Fabricius).	Archives of Phytopathology and Plant Protection, (2009) Vol. 42, No. 10, pp. 903-914. http://www.tandfonline.com/loi/gapp20. CODEN: APPZAJ. ISSN: 0323-5408. E-ISSN: 1477-2906.	Not determined to be relevant
Pedroso de Moraes, Carla; Foerster, Luis Amilton	2012	Toxicity and residual control of *Plutella xylostella* L. (Lepidoptera: Plutellidae) with *Bacillus thuringiensis* Berliner and insecticides	Ciencia Rural (2012), 42(8), 1335-1340 CODEN: CIRUEP; ISSN: 0103-8478	Not determined to be relevant
Ratanasatien, F.; Ketunuti, U.; Tantichodok, A.; Faisan Ratanasatien; Uthai	2007	Positioning of biopesticides in Thailand.	Proceedings of the 6th Pacific Rim Conference on the biotechnology of *Bacillus thuringiensis* and its environmental impact, Victoria, BC, Canada, 30 October - 3 November, 2005 (2007), pp. 100-107, 16 refs. ISBN: 978-2-9810223-0-1 Published by: National Sciences and Engineering Research Council of Canada	Not determined to be relevant

Author(s)	Year	Title	Source	Reason(s) for not including this study in the dossier (reliability criteria, point 3 above)
Ketunuti; Achara Tanticho-dok Editor(s): Cote, J. C.; Otvos, I. S.; Schwartz, J. L.; Vincent, C.			(NSERC), Ottawa Conference: Proceedings of the 6th Pacific Rim Conference on the biotechnology of *Bacillus thuringiensis* and its environmental impact, Victoria, BC, Canada, 30 October - 3 November, 2005.	
Silva, Maria C.; Siqueira, Herbert A. A. [Reprint Author]; Marques, Edmilson J.; Silva, Liliane M.; Barros, Reginaldo; Lima Filho, Jose V. M.; Silva, Suzana M. F. A.	2012	*Bacillus thuringiensis* isolates from northeastern Brazil and their activities against *Plutella xylostella* (Lepidoptera: Plutellidae) and *Spodoptera frugiperda* (Lepidoptera: Noctuidae).	Biocontrol Science and Technology, (2012) Vol. 22, No. 5, pp. 583-599. http://www.tandfonline.com/loi/cbst20. ISSN: 0958-3157. E-ISSN: 1360-0478.	Not determined to be relevant
Vicidomini, S.; Bernardo, U.; Laudonia, S.; Sannino, L.	2006	Lethal and sublethal effects *of Bacillus thuringiensis* Berliner ssp. *aizawai* on *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) larvae in extended laboratory tests. *Effetti letali* e subletali di *Bacillus thuringiensis* Berliner ssp. aizawai su larve di *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) in	Bollettino del Laboratorio di Entomologia Agraria "Filippo Silvestri" (2006), Volume 61, pp. 53-61, 32 refs. ISSN: 0304-0658 Published by: Dipartimento di Entomologia e Zoologia Agriaria dell'Universita di Napoli Frederico II, Portici	Not determined to be relevant

Author(s)	Year	Title	Source	Reason(s) for not including this study in the dossier (reliability criteria, point 3 above)
		prove di laboratorio esteso.		
Wanapaisan, P.; Chumsakul, O.; Panbangred, W. [Reprint Author]	2013	Enhanced Cry1Da production in *Bacillus thuringiensis* by driving expression from the sigma(E)-dependent BtI promoter.	Journal of Applied Microbiology, (SEP 2013) Vol. 115, No. 3, pp. 859-871. http://onlinelibrary.wiley.com/journal/10.1111/(ISSN)1365- 2672. ISSN: 1364-5072. E-ISSN: 1365-2672.	Not determined to be relevant

Conclusions

A literature search was conducted to obtain public domain references relating to the active substance *Bacillus thuringiensis aizawai* ABTS-1857 (Bta). A number of databases were searched; the date span was January 2005 to August 2016.

In total, 519 unique summary records were retrieved from bibliographic databases and were screened by expert reviewers and grouped into two categories according to their likely relevance after rapid assessment of titles and, when available, abstracts. After the intial screen 485 records were deemed to be not relevant. Full text assessments were made on the remaining 34 records to determine both relevance and reliability, and following these reviews 26 references were excluded leaving 8 references for inclusion in the dossier.

Comment by RMS

Note that the literature search was performed for all the aspects, however only the sections relevant for ecotoxicology are presented here.
The RMS is of opinion that the Cry toxins and at least insects (i.e. non-target arthropods, bees and bumblebees, should have been included in the search terms.
In general, the RMS agrees with the search terms used for the identification of peer-reviewed and public literature relevant for the active substance. Also RMS agrees with the choice of the applicant to exclude the papers presented in table 6.

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner

			not				
IIM 8.1/01	[redacted]	*1991 a*	*ABG-6305: An avian oral pathogenicity and toxicity study in the bobwhite Report No. 161-117 Source: [redacted] GLP Not published* ***PREVIOUSLY EVALUATED. IN DAR (2008)***	*Y*	*N*	*N/A*	*VBC*
IIM 8.1/02	[redacted]	*1991 b*	*ABG-6305: An avian oral pathogenicity and toxicity study in the mallard Report No. 161-118 Source: [redacted] GLP Not published* ***PREVIOUSLY EVALUATED. IN DAR (2008)***	*Y*	*N*	*N/A*	*VBC*
IIM 8.2.1/01	[redacted]	*1991 a*	*Acute toxicity of ABG-6305 to the rainbow trout On-corhynchus mykiss*	*Y*	*N*	*N/A*	*VBC*

			Report No. 9107A *Source:* [redacted] *GLP* *Not published* ***PREVIOUSLY EVALUATED. IN DAR (2008)***				
IIM 8.2.1/02	[redacted]	*1993*	*Xentari Technical Powder (ABG-6305) – Infectivity and pathogenicity to rainbow trout (Oncorhynchus mykiss) during a 20-day static renewal test* *Report No. 93-6-4837* *Source:* [redacted] *Inc.* *GLP* *Not published* ***PREVIOUSLY EVALUATED. IN DAR (2008)***	*Y*	*N*	*N/A*	*VBC*
IIM 8.2.1/03	[redacted]	*1995 a*	*Xentari technical powder (ABG-6305) – toxicity to rainbow trout (Oncorhynchus mykiss) during a 30-day static renewal test*	*Y*	*N*	*N/A*	*VBC*

			Report No. 95-7-5977 *Source:* [redacted] *GLP* *Not published* ***PREVIOUSLY EVALUATED. IN DAR (2008)***				
IIM 8.2.2/01	*Collins, M.K.*	*1995 b*	*ABG-6305 – Toxicity to water fleas (Daphnia magna) under static renewal conditions.* *Report No. 95-5-5855* *Source: Springborn Laboratories Inc.* *GLP* *Not published* ***PREVIOUSLY EVALUATED. IN DAR (2008)***	*N*	*N*	*N/A*	*VBC*
IIM 8.2.2/02	*Boeri, R.L.*	*1991 b*	*Chronic toxicity of ABG-6305 to the daphnid Daphnia magna* *Report No. 90162-A* *Source: Resource Analysts Inc.* *GLP*	*N*	*N*	*N/A*	*VBC*

			Not published ***PREVIOUSLY EVALUATED. IN DAR (2008)***				
IIM 8.2.2/03	*Ward, T.J., Kowalski, P.L., Boeri, R.L.*	*1993*	*Acute Toxicity of Components of ABG-6305 to the Daphnid Daphnia magna - addendum to MRID 41974802 Report No. 123-AB Source: T.R. Wilbury Laboratories Inc. GLP Not published* ***PREVIOUSLY EVALUATED. IN DAR (2008)***	*N*	*N*	*N/A*	*VBC*
IIM 8.2.2/04	*Collins, M.K.*	*1995 c*	*Comparative toxicity test exposing Daphnids (Daphnia magna) to XenTari technical powder produced by two different recovery processes Report No. 95-2-5717 Source: Springborn Laboratories*	*N*	*N*	*N/A*	*VBC*

			Inc. *GLP* *Not published* ***PREVIOUSLY EVALUATED. IN DAR (2008)***				
IIM 8.2.2/05	*Feng, S.Y.*	*1966*	*Experimental bacterial infections in the oyster Crassostrea virginica* *Journal of Invertebrate Pathology. Vol. 8 pp 505-511 (1966)* *Non-GLP* *Published* ***PREVIOUSLY EVALUATED. IN DAR (2008)***	*N*	*N*	*N/A*	*N/A*
IIM 8.3/01	*Kirkland, R.L.*	*1991 a*	*The effect of Bacillus thuringiensis, ABG-6305 technical powder, on the honey bee, Apis mellifera L.* *Report No. CAR 19-690* *Source: California Agricultural Research, Inc.* *GLP* *Not published* ***PREVIOUSLY EVALUATED.***	*N*	*N*	*N/A*	*VBC*

			IN DAR (2008)				
IIM 8.3/02	Gray, J.	2016	BTC Aizawi: Honey bee (*Apis mellifera*) larval toxicity test, single exposure Report No. XY58HS Envigo CRS, Ltd. GLP Unpublished **SUBMITTED FOR THE PURPOSE OF RENEWAL**	N	Y	New data submitted for first time	VBC
IIM 8.4/01	*Nelson, R.D.*	*1991 a*	*The effect of Bacillus thuringiensis, ABG-6305 technical powder, on the predatory mite Metaseiulus occidentalis (Nesbit) and their host prey the twospotted spider mite Tetranychus urticae (Koch).* *Report No. 91.042* *Source: Plant Sciences, Inc.* *GLP* *Not published* ***PREVIOUSLY EVALUATED. IN DAR (2008)***	*N*	*N*	*N/A*	*VBC*
IIM 8.4/02	*Kirkland, R.L.*	*1991 b*	*The effect of Bacillus thuringiensis, ABG-6305 technical*	*N*	*N*	*N/A*	*VBC*

			powder, on the insect egg parasitoid (Trichogramma pretiosum Riley) *Report No. CAR 104L-91* *Source: California Agricultural Research GLP* *Not published* ***PREVIOUSLY EVALUATED. IN DAR (2008)***				
IIM 8.4/03 (supplemental report)	*Castellon, A.*	*1991 c*	*The effect of Bacillus thuringiensis, ABG-6305 technical powder, on the insect egg parasitoid (Trichogramma pretiosum Riley)- supplemental report* *Report No. CAR 104L-91* *Source: California Agricultural Research GLP* *Not published* ***PREVIOUSLY EVALUATED. IN DAR (2008)***	*N*	*N*	*N/A*	*VBC*
IIM 8.4/04	*Palmer, S.J., Beavers, J.B.*	*1993*	*Xentari technical powder (ABG-6305): a*	*N*	*N*	*N/A*	*VBC*

			dietary pathogenicity and toxicity study with the lady-bird beetle (Hippodamia convergens). Report No. 161-126A Source: Wildlife International, Ltd. GLP Not published ***PREVIOUSLY EVALUATED. IN DAR (2008)***				
IIM 8.4/05	*Nelson, R.D.*	*1991 b*	*The effect of Bacillus thuringiensis, ABG-6305 technical powder, on the common green lacewing Chrysoperla carnea (Stephens). Report No. 91.043 Source: Plant Sciences, Inc. GLP Not published* ***PREVIOUSLY EVALUATED. IN DAR (2008)***	*N*	*N*	*N/A*	*VBC*
IIM 8.5/01	*Smirnoff, W.A. Heimpel, A.M.*	*1961*	*Notes on the pathology of Bacillus thuringiensis var.*		*N*		*--*

			thuringiensis Berliner for the earthworm Journal of Insect Pathology. Vol. 3 pp 403-408 Not GLP; Published ***PREVIOUSLY EVALUATED. IN DAR (2008)***				
IIM 8.5/02	Sindermann, A.B., Porch, J.R., Krueger, H.O.	2006	*Bacillus thuringiensis* subspecies *aizwai*, Strain ABTS-1857 Technical Powder: A pathogenicity study with the earthworm in an artificial soil substrate Report No. 529-110 Source: Wildlife International, Ltd. GLP Not published **SUBMITTED FOR THE PURPOSE OF RENEWAL**	N	Y	New data submitted for first time	VBC