Renewal

Assessment Report

Bacillus thuringiensis subsp. aizawai strain GC-91

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

July 2018

Rapporteur Member State: The Netherlands

Co-Rapporteur Member State: Germany

Version history

When	What
July 2018	Initial RAR

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Introduction

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 Subsequently Regulation (EC) No 1107/2009 repealed and replaced Directive 91/414/EEC and the active substance Bta GC-91, was deemed to be approved under that Regulation and included in the Annex to Regulation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

The approval of Bta GC-91 under the Regulation (EC) No 1107/2009 expires 30 April 2019. In accordance with the same Regulation the original notifier Mitsui AgriScience International SA/NV has filed to the Commission an application for the renewal of the approval of the active substance Bta GC-91 on 30 April 2016. In accordance with Regulation (EU) 2016/183 the notifier is submitting to the designated RMS The Netherlands, the co-RMS Germany as well as to EFSA and the Commission a dossier for renewal of Bta GC-91 considering the deadline stated in SANTE-2016-10616–rev. 3. As the manufacturing process of Bta GC-91 has not been changed since original approval, all data submitted for the original approval of the strain are considered fully applicable for the current evaluation. The information from the previous DAR (May 2007) and DAR addendum (February 2013) is included along with new data which resulted during the literature search.

Mode of action

Bta GC-91 is a transconjugant strain originating from a Bta and a Bt subsp. *kurstaki* strain. Bta in general occurs ubiquitous in soils on plants as well as in infested insects. Bta acts highly specific against insect species of the order Lepidoptera and is not expected to have any harmful effects on beneficials and other non-target species of other insect orders. The insecticidal activity of Bta is mainly attributed

to spore bound insecticidal pro-toxins (Cry toxins) which are ingested by the target pests and activated under alkaline conditions in the midgut of the larvae.

Metabolites

Analysis of five batches GC-91 showed that no microbial pathogens of toxicological concern for human and animal health were detected. Quality tests did not reveal the presence of toxigenic pathogens producing nameable amounts of metabolites of toxicological concern for human and animal health. MPCA does not contain any additives.

Representative uses and formulation

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

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

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

Crop	F G or I	Pest	Application			Application rate		
			Method / Kind	Growth stage of crop	Max. number (min. interval between applications) a) per use b) per crop/ season	Kg product / ha a) max. rate per appl. b) max. total rate per crop/season	g as/ha IU/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max

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

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

B.9.1 Effects on birds

B.9.1.1 Toxicity to birds

ACTIVE INGREDIENT (MPCA)

DATA FROM THE PREVIOUS DAR

Reference: **KMA 8.1/01**	[redacted] (1990a), CGA-237218 Technical (GC-91): An avian oral pathogenicity and toxicity study in the bobwhite. Unpublished Report No. 108-308, 20.12.1990
Guideline:	FIFRA Guideline No. 154A-16
GLP:	Yes
Material and methods:	The study was conducted during the period 26.02.1990 to 28.03.1990, by [redacted] The test material used was CGA 237218 Technical (GC-91) (conc. of a.i.: 10.6×10^{10} CFU/g; batch code: P90

	3001, expiry date: May 1992). Northern bobwhite (Colinus virginianus) were 14 days of age at the start of the test. All birds were provided with a game bird ration and water ad libitum during acclimation and during the test. Two groups with 5 birds each served as negative controls and were administered distilled water (1% v/w) by oral gavage daily for 5 days. For the attenuated control two groups with 5 birds each received oral doses of heat deactivated test material at 3333 mg/kg b.w. per day for 5 days. The remaining 6 groups of 5 birds received test material at 3333 mg/kg or approximately 3.53 x 10^{11} CFU/kg b.w. per day for 5 days. Average ambient temperature was 24°C ± 4°C with an average relative humidity of 29% ± 12%. The photoperiod was 16 hours of light. All birds were observed at least twice daily. Body weights were recorded individually prior to dosing on days 0, 1, 2, 3 and 4 and on days 11, 18, 25 and 30. Average feed consumption was measured for day 0-4, 4-11, 11-18, 18-25 and 25-30.
Micro-organism:	CGA 237218 Technical (GC-91)
Test species:	Northern bobwhite (Colinus virginianus)
Number of test animals:	6 groups of 5 birds, two groups with 5 birds each served as negative controls, two groups with 5 birds were the attenuated control.
Treatments:	The treated groups received test material at 3333 mg/kg or approximately 3.53 x 10^{11} CFU/kg b.w. per day
Duration:	30 days (5 of treatment)
Test conditions:	Average ambient temperature was 24°C ± 4°C with an average relative humidity of 29% ± 12%. The photoperiod was 16 hours of light.
Deviations from guideline	Samples of the test solutions were not taken to verify test dosage concentrations
Endpoint:	No mortalities occurred among the treated groups and in the negative control group. Due to the timing and nature of the clinical signs observed, they were considered to be unrelated to treatment. When compared to the negative control and attenuated control, there was no effect on body weights or feed consumption among the treatment group at any time interval. Necropsy of the treatment groups revealed an old head lesion and three treated birds were noted with feather loss on the head.
Observations:	The LD_{50} value was estimated to be > 3333 mg test material/kg or 3.53 x 10^{11} CFU/kg b.w. per day.

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	> 3333 mg test material/kg 3.53 x 10^{11} CFU/kg b.w./day
Infectivity / Pathogenicity	Not pathogenic Infectivity not determined in studies

Comments RMS:

The study was previously evaluated in the DAR (March 2008) 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 guideline was made.

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

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.

Reference: **KMA 8.1/02**	[redacted] (1990b), CGA-237218 Technical (GC-91): An avian oral pathogenicity and toxicity study in the mallard. Unpublished Report No. 108-309, 11.06.1990
Guideline:	FIFRA Guideline No. 154A-16
GLP:	Yes
Material and methods:	The study was conducted during the period 29.01.1990 to 28.02.1990, by [redacted] The test material used was CGA 237218 Technical (GC-91) (conc. of a.i.: 10.6 x 10^{10} spores/g; batch code: P90 3001, expiry date: May 1992). Mallard (Anas platyrhynchos) were 21 days of age at the start of the test. All birds were provided with a game bird ration and water ad libitum during acclimation and during the test. Two groups with 5 birds each served as negative controls and were administered distilled water (1% v/w) by oral gavage daily for 5 days. For the attenuated control two groups with 5 birds each received oral doses of heat deactivated test material at 3333 mg/kg b.w. per day for 5 days. The remaining 6 groups of 5 birds received test material at 3333 mg/kg or approximately 3.53 x 10^{11} CFU/kg b.w./day for 5 days. Average ambient

	temperature was 21°C ± 3°C with an average relative humidity of 40% ± 13%. The photoperiod was 16 hours of light. All birds were observed at least twice daily. Body weights were recorded individually prior to dosing on days 0, 1, 2, 3 and 4 and on days 11, 18, 25 and 30. Average feed consumption was measured for day 0-4, 4-11, 11-18, 18-25 and 25-30.
Micro-organism:	Bta Technical grade material CGA 237218 (GC 91)
Test species:	Mallard (Anas platyrhynchos)
Number of test animals:	5 groups of 6 birds were used as treated birds. Two control groups were performed: a negative control and an attenuated control. In the first case, two groups with 5 birds each served as negative controls and were administered distilled water (1% v/w) by oral gavage daily for 5 days. For the attenuated control two groups with 5 birds each received oral doses of heat deactivated test material at 3333 mg/kg b.w. per day for 5 days
Treatments:	3333 mg/kg b.w. per day for 5 days
Duration:	30 days (5 of treatment)
Test conditions:	Average ambient temperature was 21°C ± 3°C with an average relative humidity of 40% ± 13%.
Deviations from guideline	Samples of the test solutions were not taken to verify test dosage concentrations
Endpoint:	The LD_{50} value was estimated to be > 3333 mg test material/kg or 3.53 x 10^{11} CFU/kg b.w./day
Observations:	Gross necropsy on all other treated birds was not remarkable. No mortalities occurred among the treated groups and in the negative control group. Feather-picking was noted in one of the treatment birds. Two treated birds were noted with hanging primaries. When compared to the negative control and attenuated control, there was no effect on body weights or feed consumption among the treatment group at any time interval. Necropsy of the treatment groups revealed a bumble foot, which was not considered to be treatment related.

Results:

A summary of endpoints is given in the table below.

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

Test species	Mallard duck *(Anas platyrhynchos)*
Toxicity	> 3333 mg test material/kg

	3.53 x 10^{11} CFU/kg b.w./day
Infectivity / Pathogenicity	Not pathogenic Infectivity not determined in studies

Comments and conclusion RMS:

The study was previously evaluated in the DAR (March 2008) 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 current guideline was made.

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

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 (MCPA)

No study submitted. The following information was included in the original version of the DAR:
"The Bta strain GC-91 does not produce metabolites toxic for human health or the environment. This is confirmed by the quality control tests during the fermentation and production process, respectively. The working vials will be tested for the amount of ß-exotoxins in the fermentation broth by HPLC analysis, fly test and mice injection. Furthermore, after the fermentation process the spores will be spray dried while the nutrient broth is discarded. If any metabolites would occur, they would be removed from the Technical Powder consequently (Chen & Hargrove, 2003)".

Insectivorous and omnivorous birds can be exposed to the insecticidal metabolites that are formed in situ. According to the information provided in Volume 1, it was demonstrated that Bta GC-91 can produce Cry1Ac, Cry1C, Cry1D and Cry2A insecticidal proteins. Apart from the Cry proteins several other insecticidal proteins produced by Bt and are contributing to their mode of action have been described as well (vegetative insecticidal proteins VIP, cytolytic proteins Cyt etc.). Beta-exotoxins, are considered to have toxic properties but were shown not to be produced by commercial Btk strains. The literature search did not provide any information regarding the toxicity of the metabolites to birds.

New data 2016

Data provided for first approval are considered acceptable to cover current requirements and therefore, no substantial new information is submitted for renewal of the strain according to Regulation (EC) No 1107/2009.

The literature search covering the last 10 years and focussing to obtain references on possible toxicity or pathogenicity of Bta to birds did not provide any relevant information.

Due to strain specific data presented above and available knowledge about *Bacillus thuringiensis* subsp. *aizawai* in general it can be concluded that Bta GC-91 is not toxic, pathogenic or infective in birds.

B.9.1.2 Infectiveness to birds

It is unlikely that the MCPA exhibits infective behavior in birds as no signs of pathogenicity were observed at the high doses tested.

B.9.1.3 Pathogenicity to birds

No pathogenic effects were observed in the above studies.

B.9.1.4 Summary of the studies on birds on toxicity, infectiveness and pathogenicity

Table 9.1.4.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 (5 days of treatment)	LC50: >3333 mg/kg >3.53 x 10^{11} CFU/kg b.w./day	LC50: >3333 mg/kg >3.53 x 10^{11} CFU/kg b.w./day	No signs of mortality.	[redacted] ,1990a,
Mallard duck *(Anas platyrhynchos)*	30 days (5 days of treatment)	3333 mg/kg or approximately 3.53 x 10^{11} CFU/kg b.w./day	3333 mg/kg or approximately 3.53 x 10^{11} CFU/kg b.w./day	No signs of mortality.	[redacted] 1990b
INFECTIVENESS					
Not determined.					
PATHO-GENICITY					
Bobwhite quail *(Colinus virginianus)*	30 days	LC50: >3333 mg/kg >3.53 x 10^{11} CFU/kg	LC50: >3333 mg/kg >3.53 x 10^{11} CFU/kg	No signs of pathogenicity.	[redacted] 1990a,

Species	Test duration	Dose range	Results/ Endpoint	Observations	Reference
		b.w./day	b.w./day		
Mallard duck *(Anas platyrhynchos)*	30 days	3333 mg/kg or approximately 3.53 x 10^{11} CFU/kg b.w./day	3333 mg/kg or approximately 3.53 x 10^{11} CFU/kg b.w./day	No signs of pathogenicity.	[redacted] 1990b

B.9.2 Effects on aquatic organisms

B.9.2.1 Effects on fish

Information on toxicity to the rainbow trout (*Onchorhynchus mykiss*) and on Sheepshead minnow (*Cyprinodon variegatus*) from the previous DAR (2012) are reported.

B.9.2.2 Toxicity to fish

ACTIVE INGREDIENT (MPCA)

DATA FROM THE PREVIOUS DAR

Reference: **KMA 8.2.1/01**	[redacted](1991a), CGA-237218 Technical material – infectivity and pathogenicity to rainbow trout (Oncorhynchus mykiss) during a 32-day static renewal test. Unpublished Report No. 90-6-3363, 28.06.1991
Guideline:	FIFRA Guideline 154-19 Deviations: 1. Temperature ranged from 9-16°C instead of 12°C ± 2°C
GLP:	Yes
Material and methods:	The study was conducted during the period 19.04.1990 to 21.05.1990, by [redacted] CGA 237218 Technical (GC-91) (conc. of a.i.: 10.6 x 10^{10} CFU/g; batch number: P902001). Rainbow trout (Oncorhynchus mykiss) are gradually acclimated to the test conditions for at least 14 days prior to testing. During the 32 day test period the fish were fed daily at a growth-promoting rate of approx. 3% of body weight. The test was performed under static renewal conditions. Fresh test solutions are prepared twice weekly. The concentration of CFU/L in the control and exposure solutions was monitored at each renewal period. Pro-

	cedures used to determine the concentration of CGA-237218 were in accordance with Apha et al. (1989)[1]. Preliminary studies were performed to determine the maximum concentration of CGA-237218 achievable without adverse effects on water quality and the amount of CGA-237218 which could be adhered to food and remain associated for one hour following addition to water. The food was mixed with the MPCA at a nominal concentration equivalent to 1.52 x 10^{10} CFU per gram of food and was provided to the test organisms. The test material was incorporated into the food in batches of 38.5 g by mixing measured quantities of 5.519 g of test material (5.85 x 10^{11} CFU), with approx. 3 g fish oil and 30 g of dry fish food. During the preliminary study, rainbow trout were exposed under static conditions to nominal concentrations equivalent to 3.90 x 10^{10} CFU/L, 3.90 x 10^{9} CFU/L and 1.0 x 10^{9} CFU/L. The highest concentration was selected as the single nominal treatment concentration for the definitive test. This concentration appeared to be the maximum possible aquatic exposure under these conditions. Sixty fish were distributed to the test treatment and control aquaria (three replicates of ten fish each) to initiate the definitive exposure. Observations of mortality, abnormal behaviour and gross pathogenic response were made twice throughout the study. At test termination, fish were examined for internal and external lesions, necrosis, or tumors.
Micro-organism	Bta Technical grade material CGA 237218 (GC 91)
Test species:	Rainbow trout (Onchorhyncus mykiss)
Number of test animals:	Sixty fish were distributed to the test treatment and control aquaria (three replicates of ten fish each) to initiate the definitive exposure
Treatments:	During the preliminary study, rainbow trout were exposed under static conditions to nominal concentrations equivalent to 3.90 x 10^{10} CFU/L, 3.90 x 10^{9} CFU/L and 1.0 x 10^{9} CFU/L.
Duration:	32 days
Test conditions:	Not clearly indicated
Deviations from guideline	Temperature ranged from 9-16°C instead of 12°C ± 2°C.
Endpoint:	Measured concentrations of the MPCA in the aqueous exposure solutions were ranging from 1.4 x 10^{10} to 2.7 x 10^{10} CFU/L. Measurement of the exposure solutions resulted in a mean measured concentration of 2.0 x 10^{10} CFU/L (51% of the nominal treatment level) due to the tendency of the bacterial spores to form aggregates in solution. Analysis of old solutions indicated

[1] APHA, AWWA, WPCF. (1989) Standard methods for the examination of water and wastewater. 16th Edition, Washington, DC, 1288 pp.

	that the viability of the material did not diminish greatly between renewals. Control solution concentration of CGA-237218 fell at least three orders of magnitude lower than test concentrations. At least 98.6% of the test material adhered to the food and was available for consumption by the fish. The mean dietary concentration was estimated at 1.1 x 10^9 CFU/g, providing an additional route of exposure to the fish. The pH and dissolved oxygen of the test solutions were unaffected by the concentration of CGA-237218 tested. Daily observations did not reveal abnormal behaviour of the treatment fish compared to the control fish. One fish (3%) died in the treatment group on day 25, while no fish died in the control. No colonies of CGA-237218 were recovered from the tissue sample of the single dead fish. Microbial counts revealed considerable difference between GCA-237218 concentrations in the gill of the potentially affected fish and the unaffected fish. Microbiological culturing of the affected tissues showed that, while CGA-237218 was present in the gill, its presence did not constitute an infection. No other abnormalities were noted.
Observations:	Exposure of rainbow trout through aqueous and dietary routes caused no adverse effects to the fish based on parameters of survival, infectivity and/or pathogenicity. The LC_{50} value at 32 days was estimated to be > 2.0 x 10^{10} CFU/L.

Results:

A summary of endpoints is given in the table below.

Table B.9.2.1.a: Toxicity effects/ Infectivity / Pathogenicity of the MPCA to fish

Test species	Rainbow trout (*Onchorhyncus mykiss*)
Toxicity	The LC_{50} value at 32 days was estimated to be > 2.0 x 10^{10} CFU/L (mean measured concentration).
Infectivity / Pathogenicity	No signs of toxicity, pathogenicity or infectivity. Increased concentration of cfu were measured in the gills after necropsy, but did not contstitute infection.

Comments RMS:

The study was previously evaluated in the DAR (March 2008) 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. This is considered acceptable, however a comparison to the current guideline was made.

As reported in the study itself, the weight of the fish (0.36 – 0.97g, mean 0.64g) was slightly lower for some fish than the required range (0.5 – 5g) in the interest of using fish of the same age. This is not considered to have influenced the results particularly as the length requirement has been adhered to.

No signs of treatment related pathogenicity, toxicity or infectivity were observed.

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

Reference: **KMA 8.2.1/02**	[redacted](1991b), (CGA-237218) Technical material – infectivity and pathogenicity to sheephead minnow (Cyprinodon variegatus) during a 30-day static renewal test. Unpublished Report No. 90-8-3439, 28.06.1991
Guideline:	FIFRA Guideline 154-21
GLP:	Yes
Material and methods:	The study was conducted during the period 19.06.1990 to 19.07.1990, by [redacted] CGA 237218 Technical (GC-91) (conc. of a.i.: 10.6 x 10^{10} CFU/g; batch number: P903001). Sheepshead minnow (Cyprinodon variegatus) are gradually acclimated to the test conditions for at least 14 days prior to testing. During the 30 day test period the fish were fed daily at a growth-promoting rate of approx. 2% of body weight. The test was performed under static renewal conditions. Fresh test solutions are prepared twice weekly. The concentration of CFU/L in the control and exposure solutions was monitored at each renewal period. Procedures used to determine the concentration of CGA-237218 were in accordance with Apha et al. (1989)[2]. Preliminary studies were performed to determine the maximum concentration of CGA-237218 achievable without adverse effects on water quality and the amount of CGA-237218 which could be adhered to food and remain associated for one hour following addition to water. The food was mixed with the MPCA at a nominal concentration equivalent to 1.56 x 10^{10} CFU per gram of food and was provided to the test organisms. The test material was incorporated into the food by mixing measured quantities of 5.52 g of test material (5.85 x 10^{11} CFU), with 1.96 g fish oil and 30 g of dry fish food. During the preliminary study, sheepshead minnow were exposed under static conditions to nominal concentrations equivalent to 3.90 x 10^{10} CFU/L, 3.90 x 10^{9} CFU/L and 1.0 x 10^{9}

[2] AHA, AWWA, WPCF. (1989) Standard methods for the examination of water and wastewater. 16th Edition, Washington, DC, 1288 pp.

	CFU/L. Following 96 hours of exposure (the longest period between renewals), the dissolved oxygen concentration in the highest concentration had dropped below 60% of saturation. Although no mortality was observed at that concentration, the next highest concentration (3.90 x 10^9 CFU/L) was selected as the single nominal treatment concentration for the definitive test in order to maintain acceptable water quality. Sixty fish were distributed to the test treatment and control aquaria (three replicates of ten fish each) to initiate the definitive exposure. Observations of mortality, abnormal behaviour and gross pathogenic response were made twice throughout the study. At test termination, fish were examined for internal and external lesions, necrosis, or tumors.
Micro-organism	Bta - CGA 237218 Technical (GC-91)
Test species:	Sheepshead minnow (Cyprinodon variegatus)
Number of test animals:	Sixty fish were distributed to the test treatment and control aquaria (three replicates of ten fish each)
Treatments:	Exposure at 3.90 x 10^9 CFU/L
Duration:	30 days
Test conditions:	Not clearly indicated
Deviations from guideline	Deviations: 1. Temperature ranged from 17-24°C instead of 22°C ± 2°C
Endpoint:	Measured concentrations of the MPCA in the aqueous exposure solutions were ranging from 4.8 x 10^8 to 1.1 x 10^{10} CFU/L. Measurement of the exposure solutions resulted in a mean measured concentration of 2.1 x 10^9 CFU/L (53.8% of the nominal treatment level) due to the tendency of the bacterial spores to form aggregates in solution. Analysis of old solutions indicated that CGA-237218 concentrations generally decreased somewhat during the period between renewals. Control solution concentration of CGA-237218 consistently fell at least three orders of magnitude lower than test concentrations. At least 98.6% of the test material adhered to the food and was available for consumption by the fish. The mean dietary concentration was estimated at 9.1 x 10^8 CFU/g, providing an additional route of exposure to the fish. The pH and dissolved oxygen of the test solutions were unaffected by the concentration of CGA-237218 tested. During the 30-day study, no mortality was observed in either the treatment or control. Results of the necropsy conducted at test termination confirmed the absence of inflammatory responses or necrosis. Overall, no evidence of infectivity or pathogenicity was apparent in the course of this study.

Observations:	Exposure of sheepshead minnow through aqueous and dietary routes caused no adverse effects to the fish based on parameters of survival, infectivity and/or pathogenicity. The LC_{50} value at 30 days was estimated to be > 2.1 x 10^9 CFU/L

Results:

A summary of endpoints is given in the table below.

Table B.9.2.1.b: Toxicity effects/ Infectivity / Pathogenicity of the MPCA to fish

Test species	Sheepshead minnow (*Cyprinodon variegatus*)
Toxicity	The LC_{50} value at 30 days was estimated to be > 2.1 x 10^9 CFU/L (mean measured concentration)
Infectivity / Pathogenicity	No signs of pathogenicity or infectivity.

Comments and conclusion RMS:

The study was previously evaluated in the DAR (March 2008) 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. This is considered acceptable, however a comparison to the current guideline was made.

As reported in the study itself, the weight of the fish (0.18 – 1.1 g, mean 0.42 g) was slightly lower for some fish than the required range (0.5 – 5g) in the interest of using fish of the same age. This is not considered to have influenced the results particularly as the length requirement has been adhered to.

No signs of treatment related pathogenicity, toxicity or infectivity were observed.

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

Toxin/metabolite from microbial pest control agent (MPCA)

No study submitted. The following information was included in the original version of the DAR:
"The Bta strain GC-91 does not produce metabolites toxic for human health or the environment. This is confirmed by the quality control tests during the fermentation and production process, respectively. The working vials will be tested for the amount of ß-exotoxins in the fermentation broth by HPLC analysis, fly test and mice injection. Furthermore, after the fermentation process the spores will be spray dried while the nutrient broth is discarded. If any metabolites would occur, they would be removed from the Technical Powder consequently (Chen & Hargrove, 2003)".

According to the information provided in Volume 1, it was demonstrated that Bta GC-91 can produce Cry1Ac, Cry1C, Cry1D and Cry2A insecticidal proteins. Apart from the Cry proteins several other in-

secticidal proteins produced by Bt and are contributing to their mode of action have been described as well (vegetative insecticidal proteins VIP, cytolytic proteins Cyt etc.). Beta-exotoxins, are considered to have toxic properties but were shown not to be produced by commercial Btk strains.

Considering the type of application and the mode of action, the exposure of fish to metabolites of Bta GC-91 is not considered relevant.

New data 2016

Data provided for the first approval are considered acceptable to cover current requirements. Therefore, no substantial new information is submitted for renewal of the strain according to Regulation (EC) No 1107/2009.

The literature search covering the last 10 years and focussing to target possible toxicity or pathogenicity of Bta to fish did not provide any relevant information.

Due to strain specific data presented above and available knowledge about *Bacillus thuringiensis* subsp. *aizawai* in general it can be concluded that Bta GC-91 is not toxic, pathogenic or infective in fish.

B.9.2.3 Infectiveness to fish

No signs of infectivity.

B.9.2.4 Pathogenicity to fish

No signs of pathogenicity.

B.9.2.5 Effects on freshwater invertebrates

The studies from the previous the DAR (2012) are presented below. Additions by RMS are presented in bold.

B.9.2.6 Toxicity to freshwater invertebrates

Reference: **KMA 8.2.2/01**	Collins, M.K. (1997), CGA-237218 Acute toxicity to daphnids, Daphnia magna, under static – renewal conditions. Unpublished Report No. 97-1-6842, 12.05.1997
Guideline:	EPA Guideline 154A-20
GLP:	Yes
Material and methods:	The study was conducted during the period 13.12.1996 to 23.12.1996, by Springborn Laboratories, Inc., Wareham, Massachusetts, USA. CGA 237218 Technical; batch number: BA611040; purity: 7.5%). This study was conducted in order to determine which components of the technical material are re-

	sponsible for the effects seen in previous studies on daphnids (refer to chronic toxicity study by Christensen 1991c, Report No. 90-7-3385). Daphnia magna (≤ 24 h old) were exposed to Agree technical and potential constituents (Table 3) for 10 days under static renewal conditions (days 3, 5 and 7). Two replicate test vessels containing 10 daphnids each (20 per concentration and control) were maintained for each treatment level. Exposure solution temperatures were maintained at 20±1°C. The daphnid culture area received a regulated photoperiod of 16 hours of light and 8 hours of darkness. Observations of survival and sublethal effects (e.g. lethargy) were recorded once daily. One complete set of spore enumerations for each treatment and the control was performed on one freshly prepared (day 0) and the corresponding aged solutions (day 3). A second set of partial enumerations was also performed on test days 7 and 10. **Table 3 Description and purpose of treatments** **Treatment** / **Description** / **Determines** Control / Dilution water without test material / Any adverse effects not related to Agree 1 / Agree technical material at 100 mg/L dilution water / Toxicity of everything present in the technical material 2 / Attenuated (heat-inactivated) Agree technical material at 100 mg/L dilution water / Toxicity of heat stable and other components present in the technical material (inactivates heat-labile components) 3 / Purified spore crystal complex, obtained by 6x washing of the technical material, in the amount per liter present in 100 mg/L technical solution used for Treatment 1 (56.7 mg/L dilution water) / Toxicity of isolated delta-endotoxin, the active ingredient in the technical material 4 / A fermentation broth (0.22 µm filter sterilized supernatant) solution in the amount per liter required to produce 100 mg of technical material (4.0 mL/L dilution water) / Toxicity of soluble constituents present in the fermentation broth that may be transferred to the technical material upon spray-drying 5 / An attenuated fermentation broth (0.22 µm filter-sterilized supernatant) solution in the amount per liter required to produce 100 mg of technical material (4.0 mL heat-inactivated supernatant/L dilution water) / Toxicity of soluble, heat-stable constituents present in the fermentation broth, and potential exotoxin toxicity via inactivation of heat-labile exotoxins
Micro-organism	Bta Technical grade material CGA 237218 (GC 91)
Test species:	*Daphnia magna*
Number of test animals:	Two replicate test vessels containing 10 daphnids each (20 per concentration and control) were maintained for each treatment level
Treatments:	5 treatments: Control, Attenuated control, purified spore crystal complex, fermentation broth solution, attenuated fermentation broth
Duration:	10 days
Test conditions:	Exposure solution temperatures were maintained at 20±1°C. The daphnid culture area received a regulated photoperiod of 16 hours of light and 8

	hours of darkness
Deviations from guideline	Due to non-quantifiable plate counts in two of the aged solutions, a second set of enumeration data was collected. 2. Temperature measurements were inadvertently not recorded in the aged solutions on test day 7.
Endpoint:	At test termination, immobilization among daphnids exposed to treatment 1 and 4 was 100% (Table 4). (In the DAR and the July 2012 addendum this was incorrectly reported as survival in the control and treatment 1 and 4). No immobilization or sublethal effects were observed among daphnids exposed to the control and treatments 2 and 3. Mean immobilization of 55% and sub-lethal effects were noted among daphnids exposed to treatment 5 only on the final day of the test. The heat-inactivated technical solution (treatment 2) produced no adverse effects on the test organisms, eliminating heat-stable exotoxins (e.g., beta-exotoxin) as a potentially toxic component of treatment 1. The absence of daphnid immobilization in the purified spore-crystal complex (treatment 3), indicates that the active ingredient, delta-endotoxin, does not contribute to the toxicity of the technical material to D. magna. The unattenuated supernatant solution (treatment 4), elicited the greatest toxic response of D. magna, whereas no adverse effects were observed in daphnids exposed to the attenuated supernatant solution (treatment 5) until the final day of the test. Although the supernatant contains metal salts required for bacterial growth, the fact that the toxic response in treatment 5 did not parallel that in treatment 4 rules out the possibility of metal toxicity.

Table 4 Cumulative percent of immobilized daphnids determined during the 10-day static-renewal exposure to CGA-237218

	Percent immobilization										
Treatment	**Exposure Day**										
Level	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Negative Control A	0	0	0	0	0	0	0	0	0	0	0
B	0	0	0	0	0	0	0	0	0	0	0
Mean	0	0	0	0	0	0	0	0	0	0	**0**
Treatment 1[a] A	0	0	0	0	0	50	9	100	100	100	100
B	0	0	0	0	0	20	80	100	100	100	100
Mean	0	0	0	0	0	35[b]	85[b]	100	100	100	**100**
Treatment 2[a] A	0	0	0	0	0	0	0	0	0	0	0
B	0	0	0	0	0	0	0	0	0	0	0
Mean	0	0	0	0	0	0	0	0	0	0	**0**
Treatment 3[a] A	0	0	0	0	0	0	0	0	0	0	0
B	0	0	0	0	0	0	0	0	0	0	0

Mean	0	0	0	0	0	0	0	0	0	0	**0**
Treatment 4[a] A	0	0	0	50	100	100	100	100	100	100	100
B	0	0	0	60	100	100	100	100	100	100	100
Mean	0	0	0	55[b]	100	100	100	100	100	100	**100**
Treatment 5[a] A	0	0	0	0	0	0	0	0	0	0	70
B	0	0	0	0	0	0	0	0	0	0	40
Mean	0	0	0	0	0	0	0	0	0	0	**55[c]**

[a] Test solutions were observed to be slightly cloudy with undissolved test material visible on the bottom of the vessel

[b] All of the mobile daphnids were lethargic.

[c] One of the mobile daphnids was lethargic.

Observations:

Study results suggest that one or more soluble, heat-labile components present in the supernatant are transferred to the technical material during production and are responsible for the toxicity of CGA-237218 to *Daphnia magna*. The fact that CGA 237218 tech. appears to contain heat-labile toxic components has no bearing on the ecological risk assessment. The toxicity of CGA 237218 tech. was fully characterized in previous studies and the data have been used to establish a wide margin of safety for non-target organisms.

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	No endpoint calculated, due to different purpose of study. It concluded that heat labile components from the fermentation broth are carreied over into the technical material. These components elicit the toxicity of GC-91 observed for Daphnids.
Infectivity / Pathogenicity	Not applicable, not tested.

Comments RMS:

The study was previously evaluated in the DAR 2008 and in the updated DAR (July 2012) and considered acceptable.

The study investigated the origin of the toxicity observed in other studies. It was confirmed that the toxicity is due to the presence of soluble constituents present in the fermentation broth that are transferred to the technical material. RMS would like to add that in Table 8.2.2-6 the control 4 it is stated to determine the toxicity of soluble constituents present in the fermentation broth that may be transferred to the technical material upon spray-drying. However, as the as the control 4 is not attenuated, the toxicity can only be attributed to the soluble constituents present in the fermentation broth. As the treatment 5 is attenuated, the toxicity can be attributed to heat stable components that are present in the fermentation broth and transferred to the technical material upon spray-drying. Once the fermentation is attenuated, the toxicity can be attributed to the soluble, heat-stable components (n.b. effects from the treatment 5). The toxicity cannot be attributed to beta-exotoxin as after fermentation the batches will be tested for the presence of beta-exotoxins. The study also confirms that the toxicity is not due to the delta-endotoxins (n.b. there were no effects from treatment 3). The study is considered reliable and relevant.

Reference: **KIIM 8.2.2/02**	Christensen, K.P. (1991c), (CGA-237218) Chronic toxicity to daphnids (*Daphnia magna*) under static renewal conditions. Unpublished Report No. 90-7-3385, 05.08.1991
Guideline:	FIFRA Guideline 154-20
GLP:	Yes

Material and methods:	The study was conducted during the period 11.04.1990 to 02.05.1990, by Springborn Laboratories, Inc., Wareham, Massachusetts, USA. CGA 237218 Technical (GC-91) (conc. of a.i.: 10.6 x 10^{10} CFU/g; batch number: P903001). During the 72-hour range finding test, *Daphnia magna* (≤ 24 hours old) were exposed under static renewal conditions to nominal concentrations ranging from 3.90 x 10^{10} to 1.0 x 10^{9} CFU/L. During the 21-day definitive test solutions were renewed at a frequency of three times weekly. Due to high mortality, a lower dilution series was selected for the definitive test. The nominal concentrations were 1.17 x 10^{10}, 3.51 x 10^{9}, 1.05 x 10^{9}, 3.16 x 10^{8}, 9.50 x 10^{7} CFU/L (dilution factor 0.3). Twenty daphnids were distributed to each concentration (10 daphnids per replicate). Daily during the exposure period, daphnids received a combination of trout chow and algae suspension. Test solution temperature was maintained at 18-24°C. The photoperiod during the test was 16 hours of light and 8 hours of darkness. Periodic observations on organism survival (daily) and reproduction (following day 7 of exposure period; minimum of 3 times a week), and observations on daphnid growth at test termination were recorded. Total harness and alkalinity were measured at test initiation and upon preparation of renewal solution. Temperature, pH and dissolved oxygen concentration were monitored daily in one replicate of each concentration and control. Procedures used to determine the concentration of CGA-237218 were in accordance with Apha et al. (1989).[3] Data obtained on organism survival, reproduction and growth were statistically analyzed.
Micro-organism	Bta Technical grade material CGA 237218 (GC 91)
Test species:	*Daphnia magna*
Number of test animals:	Twenty daphnids were distributed to each concentration (10 daphnids per replicate).
Treatments:	The nominal concentrations were 1.17 x 10^{10}, 3.51 x 10^{9}, 1.05 x 10^{9}, 3.16 x 10^{8}, 9.50 x 10^{7} CFU/L (dilution factor 0.3).
Duration:	21days
Test conditions:	Test solution temperature was maintained at 18-24°C. The photoperiod during the test was 16 hours of light and 8 hours of darkness
Deviations from guideline	Temperature ranged from 18-24°C instead of 20°C ± 1°C; 2. The dissolved oxygen fell below 60% saturation in the two highest test concentrations.

[3] APHA, AWWA, WPCF. (1989) Standard methods for the examination of water and wastewater. 16th Edition, Washington, DC, 1288 pp.

Endpoint:

Mean measured concentrations established averaged 51.9% of the nominal level due to the tendency of spores to form aggregates in solution. The mean measured aqueous concentrations of the test material were 5.71 x 10^9, 1.77 x 10^9, 6.24 x 10^8, 1.57 x 10^8 and 4.86 x 10^7 CFU/L. At test termination, percent survival ranged from 0% (5.71 x 10^9 CFU/L) in the highest mean measured concentration tested to 90% in the 1.57 x 10^8 CFU/L test concentration (Table 1). Survival in the control was 100%. Daphnid survival was significantly affected when daphnids were exposed to CGA-237218 at concentrations of 6.24 x 10^8 CFU/L or greater based on mean measured concentrations. Below this concentration, reproduction and growth were unaffected by the presence of CGA-237218.

Survival

Table 1. Percent survival of daphnids (*Daphnia magna*) after 21-day static renewal exposure to CGA-237218

Mean measured concentration (CFU/L)	% survival after 21 days
5.71 x 10^9	0[a]
1.77 x 10^9	0[a]
6.24 x 10^8	30[a]
1.57 x 10^8	90
4.86 x 10^7	85
Control	100

[a] significantly different from control

Reproduction data

Table 2. Cumulative number of offspring produced per female daphnid (*Daphnia magna*) after 21-day static renewal exposure to CGA-237218 (Christensen, 1991, submitted in IIM 8.3/01)

Mean measured concentration (CFU/L)	Mean cumulative offspring after 21 days
5.71 x 10^9	0[a]
1.77 x 10^9	0[a]
6.24 x 10^8	163[a]
1.57 x 10^8	127

4.86×10^7	133
Control	107

[a] Data omitted from statistical comparison of reproduction between groups due to significantly lower survival athis concentration.

Body length data

Table 3. Mean total body length data of daphnid after a 21-day static renewal exposure to CGA-237218

Mean measured Concentration (CFU/L)	Mean Body Lenght (SD)[a] (mm)
5.71×10^9	0 [b]
1.77×10^9	0 [b]
6.24×10^8	4.6 (0.12) [b]
1.57×10^8	4.7 (0.17)
4.86×10^7	4.8 (0.15)
Control	4.6 (0.22)

[a] SD = Standard deviation
[b] Data omitted from statistical comparison of lengths due to the lower survival at this concentration

Observations: The 21-day EC_{50} was estimated to be 3.24×10^8 CFU/L. The NOEC was 1.57×10^8 CFU/L and the LOEC established was 6.24×10^8 CFU/L. Based on these results, the Maximum acceptable Toxicant Concentration (MATC) was determined to be 3.13×10^8 CFU/L.

Results:

A summary of endpoints is given in the table below.

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

Test species	*Daphnia magna*
Toxicity	21-day EC_{50} (survival): 3.24×10^8 CFU/L. NOEC(length, offspring and survival): 1.57×10^8 CFU/L
Infectivity / Pathogenicity	Not tested. Signs of infectivity were not observed

	when appying the normal test procedures.

Comments and conclusion RMS:

The study was previously evaluated in the DAR 2008 and in the updated DAR (July 2012) and considered acceptable.

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 MCPA caused effects on survival, reproduction and growth in Daphnids. According to OECD 211, the temperature should be in the range of 18-22^{0}C and should not vary by more than 2^{0}C within these limits. In this case the temperature varied by 4^{0}C, with 2^{0}C above the maximum limit. The increase in temperature can affect the heart rate in daphnids. Considering that oxygen saturation also felt below the 60% in the highest concentrations, RMS is of opinion that this parameter together with the increased temperature might have had an influence on the study results.

The study is considered reliable with restrictions.

Reference: **KMA 8.2.2/03**	Collins, M.K. (1993), Infectivity and pathogenicity to daphnids (Daphnia magna) during a 21-day static renewal test. Unpublished Report No. 93-10-4968, 19.11.1993
Guideline:	EPA Guideline 154A-20
GLP:	Yes
Material and methods:	The study was conducted during the period 25.08.1993 to 15.09.1993, by Springborn Laboratories, Inc., Wareham, Massachusetts, USA. CGA 237218 Technical (GC-91) (conc. of a.i.: 9.1 x 10^{10} CFU/g; batch number: PI 102002). This study was recommended to investigate the cause of the differences between the survival of organisms in the two lowest treatment levels (90 and 85% survival) and the control observed during the previously conducted study (refer to Christensen 1991c, Report No. 90-7-3385). During the 21-day subsequent test solutions were renewed at a frequency of three times weekly. Daphnids were exposed to the following nominal concentrations of CGA-237218 Technical: 8.5 x 10^6, 2.8 x 10^7, 9.5 x 10^7, 3.15 x 10^8 and 1.05 x 10^9 CFU/L. In addition to a standard control, an attenuated control was maintained to determine if effects were related to the physical effects of CGA-237218 Technical. The attenuated control contained test material, which had been heat-killed, at a concentration equal to the concentration of the test material in the highest treatment level. Twenty daphnids were distributed to each concentration (10 daphnids per replicate). Daily during the exposure

	period, daphnids received a combination of trout chow and algae suspension. Test solution temperature was maintained at 20±1°C. The photoperiod during the test was 16 hours of light and 8 hours of darkness. Periodic observations on organism survival (at each renewal day at test termination) and reproduction (at each observation interval), and observations on daphnid growth at test termination were recorded. Daphnids were then dried at approx. 60°C for 72 hours for determination of organisms' dry weight. Biological observation and observations of the physical characteristics of the test solutions were recorded on renewal days during the exposure period. Total hardness and alkalinity were measured once weekly in alternate replicates of each concentration in the old test solution following transfer of the exposed daphnids. Temperature, pH and dissolved oxygen concentration were monitored daily in one replicate of each concentration and control. The concentration of colony forming units/L was determined in each replicate of the treatment solutions and the control solutions at test initiation and at each renewal. Verification of the test material concentration in the freshly prepared test solutions was performed at each renewal period. The concentration of material in the aged solutions was measured at test termination. Data obtained on organism survival, reproduction and growth were statistically analyzed.
Micro-organism	Bta Technical grade material CGA 237218 (GC 91)
Test species:	*Daphnia magna*
Number of test animals:	Twenty daphnids were distributed to each concentration (10 daphnids per replicate)
Treatments:	Daphnids were exposed to the following nominal concentrations of CGA-237218 Technical: 8.5 x 10^6, 2.8 x 10^7, 9.5 x 10^7, 3.15 x 10^8 and 1.05 x 10^9 CFU/L
Duration:	21 days
Test conditions:	Test solution temperature was maintained at 20±1°C. The photoperiod during the test was 16 hours of light and 8 hours of darkness
Deviations from guideline	None
Endpoint:	Measured concentrations of the test material in the freshly prepared exposure solutions averaged 63% of the nominal concentrations. The mean measured aqueous concentrations of the test material were 6.2 x 10^8, 1.7 x 10^8, 5.1 x 10^7, 1.4 x 10^7 and 8.4 x 10^6 CFU/L. Organisms survival in the control and attenuated control solutions, at the completion of the 21-day test, each averaged 95% (Table 2). There was no statistical difference between the performance (survival, reproduction and growth) of the control and atten-

uated control organisms. Thus, the two control groups were pooled. After 21 days exposure, daphnids survival, reproduction and growth was not significantly different when compared to the results of the pooled control organisms.

Table 2: Percent survival of daphnids during the 21-day static renewal exposure to CGA-237218

Mean measured concentration (CFU/L)	**Mean % survival**
Control	95
Attenuated control	95
Pooled Control	95
8.4×10^6	100
1.4×10^7	95
5.1×10^7	100
1.7×10^8	90
6.2×10^8	95

Table 3: Cumulative number of offspring produced per female daphnid (*Daphnia magna*) after 21-day static renewal exposure to CGA-237218 (Collins, 1993, submitted in in OECD Dossier, Doc M-IIM, Section 6, Point IIM 8.3)

Mean measured concentration (CFU/L)	**Mean cumulative offspring after 21 days**
Control	152
Attenuated control	156
Pooled Control	154
8.4×10^6	142
1.4×10^7	149
5.1×10^7	142
1.7×10^8	148
6.2×10^8	169[a]
[a] Statistically significant different ($p \leq 0.05$) from pooled control data.	

Observations: The 21-day EC_{50} and NOEC were estimated to be > 6.2×10^8 CFU/L. Con-

	sidering the initial and second study, and assuming that 6.2 x 10^8 CFU/L is the threshold concentration for the toxicity of CGA-237218 to *Daphnia magna*, a conservative MATC was determined to be ≤ 6.2 x 10^8 CFU/L and ≥ 1.7 x 10^8 CFU/L.

Results:

A summary of endpoints is given in the table below.

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

Test species	*Daphnia magna*
Toxicity	21-day EC_{50} > 6.2 x 10^8 CFU/L NOEC ≥ 6.2 x 10^8 CFU/L
Infectivity / Pathogenicity	No effects on pathogenicity or infectivity observed

Comments and conclusion RMS:

The study was previously evaluated in the DAR 2008 and in the updated DAR (July 2012) and considered acceptable.

The most recent guideline applicable to this test is OPPTS 885.4240 (1996). As the test was performed in 1993, the predecessor of this guideline was used. This is considered acceptable, however a comparison to the current guideline was made.

The MCPA caused no effects on survival, reproduction and growth in Daphnids at 6.2 x 10^8 CFU/L in this study whereas in the previous study by Christensen (1991c) only 30% survival was observed at this concentration. The study report explains this difference with natural variability in the sensitivity of this species. However, the RMS is of opinion that the test temperature might also have affected the fitness of the daphnids. Therefore, the results of this test should be used in the risk assessment. The 21-d NOEC from this study was determined to be 6.2 x 10^8 CFU/L. No signs of treatment related pathogenicity or infectivity was observed.

The study is considered relevant and reliable.

DAR-March 2008

Reference: **KIIM 8.2.2/04**	Christensen, K.P. (1991d), CGA-237218 technical material – infectivity and pathogenicity to grass shrimp (*Palaemonetes vulgaris*) during a 30-day static renewal test.

	Unpublished Report No. 90-6-3445, 28.06.1991
Guideline:	EPA Guideline 154-21
GLP:	Yes
Material and methods:	The study was conducted during the period 12.06.1990 to 12.07.1990, by Springborn Laboratories, Inc., Wareham, Massachusetts, USA. CGA 237218 Technical (conc. of a.i.: 1.06 x 10^{11} CFU/g; batch number: P903001). One hundred and twenty *Palaemonetes vulgaris* (20 grass shrimps per replicate) were exposed for 30 days under static renewal conditions (twice weekly) to nominal dietary concentrations of 1.58 x 10^{10} CFU/g. Prior to the definitive study, a preliminary study was conducted to determine the maximum concentration of CGA-237218 which could remain associated with the food for a period of one hour following addition to water. A commercially prepared shrimp food diet was fed to the test organisms once daily at a growth-promoting rate of approximately 6% of body weight. Control shrimp received an equal amount of food which had not been amended with the test material. No CGA-237218 was added to the culture water. Intended temperature was 20±2°C. A photoperiod of 16 hours light and 8 hours darkness was maintained. Dissolved oxygen concentrations, salinity, pH and temperature were measured daily in each replicate of the control and test concentration. Aliquots of control and treated food were assayed weekly. The concentration of CFU/L in the culture water was also monitored in one replicate vessel of treatment and control at each renewal period, altering between replicates. Observations of mortality, abnormal behaviour and gross pathogenic response (lesions or necrosis of the exoskeleton, eyes and appendages) were made twice daily. Observations on the presence of eggs were made where noted.
Micro-organism	Bta Technical grade material CGA 237218 (GC 91)
Test species:	*Palaemonetes vulgaris*
Number of test animals:	One hundred and twenty *Palaemonetes vulgaris* (20 grass shrimps per replicate)
Treatments:	Exposition under static renewal conditions (twice weekly) to nominal dietary concentrations of 1.58 x 10^{10} CFU/g
Duration:	30 days
Test conditions:	Temperature was 20±2°C. A photoperiod of 16 hours light and 8 hours darkness was maintained.
Deviations from	The measured temperature range was 18-24°C. 2. At termination, 23% mortality was observed among control organisms. Control and treatment shrimp

guideline	survival were not significantly different, an indication of the general survival rate of a grass shrimp population of this age and condition.
Endpoint:	The mean dietary concentration of the test material was 1.1 x 10^9 CFU/g, averaged 7% of the nominal level. Measurement of low levels of CGA-237218 concentrations in the culture solution indicated the presence of the test material in the water of the treated group. Some of the test material in the water may have dissociated from the food. However, it is also likely that a portion of the CGA-237218 consumed by the shrimp passed through the digestive tract unharmed and was excreted in the feces. During the 30-day study, 20% of the shrimps died in the treatment group and 23% died in the control. Survival between the two groups was not significantly different. Overall, no evidence of infectivity or pathogenicity was apparent. Feeding behaviour was observed to be normal. Two of the control shrimp and six of the treated shrimp were carrying eggs. The presence of very high concentrations of CGA-237218 in the diet of the shrimp did not adversely affect shrimp survival or growth.
Observations:	Concentration of CGA-237218 through dietary routes caused no adverse effects to shrimps, even at very high concentrations of 1.9 x 10^9 CFU/g food measured concentration. Thus, the EC_{50} is estimated to be > 1.9 x 10^9 CFU/g food.

Results:

A summary of endpoints is given in the table below.

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

Test species	*Palaemonetes vulgaris (grass shrimp)*
Toxicity	30-day EC_{50} > 1.9 x 10^9 CFU/g food NOEC ≥ 1.9 x 10^9 CFU/g food
Infectivity / Pathogenicity	No effects on pathogenicity or infectivity observed

Comments and conclusion RMS:

The study was previously evaluated in the DAR 2008 and in the updated DAR (July 2012) and considered acceptable.

The most recent guideline applicable to this test is OPPTS 885.4280 (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.

Generally, control mortality should not be higher than 20% for a test to be considered valid. The study report claims that the observed control mortality of 23% is normal for this test species, because the treatment concentration showed a similar mortality rate (20%). The study report further assumed that most deaths were related to cannibalism. The RMS considers the latter a likely scenario for this study and a reasonable explanation for the high control mortality rates, but also concludes that the test system was not appropriately designed, particularly with regard to the feeding regime. Cannibalism can be reduced by *ad libitum* feeding and mortality can be reduced to 2-3% for this species. Therefore, the claim that high mortality rates above 20% are normal and acceptable is not considered valid. Under such high mortality rates normally it is not possible to calculate reliable point estimates. As only one treatment concentration was tested, no dose response was targeted and replicates all behaved similar in terms of observed mortality, the conclusion that the test substance did not influence the test organism can still be drawn.

The measured concentrations in the water established the presence of the test material at a mean concentration of 8.0×10^6 CFU/L. According to the study authors, some of the concentration in the water may have been dissociated from food. Another part resulted from the consumed material that might have passed the digestive tract unharmed and was excreted in the feces. Considering these the endpoint based on the concentration in the water is not completely reliable.

The 21-d NOEC from this study was determined to be 1.9×10^9 CFU/g (diet exposure). No signs of treatment related pathogenicity or infectivity were observed.

The study is considered relevant and reliable with restrictions.

New data 2016

In the literature search covering the last 10 years and focussing to target possible toxicity or pathogenicity of Bta to aquatic invertebrates one article was identified, studying the susceptibility of *Daphnia similis* to microbial pest control agents including *Bacillus thuringiensis* subsp. *kurstaki.*

Reference:	Oliveira-Filho E.C., Muniz D.H., Freire I.S., Ramos F.R., Alves R.T., Jonsson C.M., Grisolia C. K., Monnerat R. G (2011): Susceptibility of non-target invertebrates to Brazilian microbial pest control agents
KIIM 8.2.2/05	Published report
	Ecotoxicology, 20, 1354-1360
Guideline:	USEPA 1996a;
	Associacao Brasileira de Normas Tecnicas, 2004
GLP:	No

Material and methods:	The study was conducted in the laboratory. Five Brazilian entomopathogenic microorganisms were tested; two were *Bacillus thuringiensis* strains of different serotypes: *B. thuringiensis* serotype *kurstaki* (Btk) and *B. thuringiensis* serotype *israelensis* (Bti). Static acute toxicity tests lasting 48 h were conducted with *Daphnia similis* (Crustacea, Cladocera). Twenty daphnids (> 5 and < 24 h old) per concentration were exposed to different dilutions of lyphilized entomopathogen spores in the assay water. The number of affected (immobilized) organisms in each beaker was determined at 24 h and 48 h and the EC_{50} values were calculated. The maximum tested concentration was 1.5×10^6 spores per mL for Btk and 1.5×10^5 spores per mL for Bti. Other experiments of the study are not presented here.
Micro-organism	*B. thuringiensis* serotype *kurstaki* (Btk) and *B. thuringiensis* serotype *israelensis* (Bti)
Test species:	*Daphnia similis*
Number of test animals:	Twenty per treatment, five per replicate, four replicates
Treatments:	Btk: Control, 5.0 x 10, 5.0 x 10^2, 5.0 x 10^3, 5.0 x 10^4, 1.5 x 10^5 spores/mL Bti: Control, 5.0 x 10^2, 5.0 x 10^3, 5.0 x 10^4, 5 x 10^5, 1,5 x 10^6 spores/mL
Duration:	48 hours
Test conditions:	20 mL beakers containing 10 ml of synthetic soft water, pH 7.4 ± 0.1, water hardness 44 mg/L as CaCO3, at 20 ± 2°C.
Deviations from guideline	Maximum tested dose was > 10^6 (USEPA's maximum theshold) and instead established by criterion of feasible visualization of the test organism , ie turbidity. No analytical verifications of the test concentrations performed.
Endpoint:	48h EC_{50}
Observations:	In the control group and in concentrations tested there was no significant change in the mobility of the test organisms after 48 h of exposure. It was not possible to observe an increase in the adverse effect related to an increase in the adverse effect related to an increment in spore concentration, and the percentage of immobilisation at the highest concentrations were lower or similar to the control. Thus, the EC_{50} at 48 h for *D. similis* can be expressed as greater than 1.5×10^6 spores per mL for Btk and as greater than 1.5×10^5 spores per mL for Bti.

Results:

A summary of endpoints is given in the table below.

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

Test species	*Daphnia similis*
Toxicity	48h EC_{50} >1.5 × 10^6 spores/mL (1.5 x 10^7 CFU/L) 48h NOEC ≥ 1.5 × 10^6 spores/mL (1.5 x 10^7 CFU/L)
Infectivity / Pathogenicity	No signs of infectivity or pathogenicity were reported.

Comments and conclusion RMS:

This published literature study was newly submitted information for the renewal of the MCPA.

An official guideline was used and the majority of the applicable criteria were fulfilled. The main caveats of the study are the lack of reported validity criteria and raw data, insufficient reporting of quality and origin of the test substance, insufficient method description, and lack of validation of exposure. The result of the study however can be used as supporting information for regulatory purposes.

Toxin/metabolite from microbial pest control agent (MPCA)

No data or information submitted for invertebrates.

B.9.2.7 Infectiveness to freshwater invertebrates

No Infectivity was observed in 21 day static renewal test by Collins, M.K. (1993).

B.9.2.8 Pathogenicity to freshwater invertebrates

No pathogenicity was observed in 21 day static renewal test by Collins, M.K. (1993).

B.9.2.9 Effects on algae growth

ACTIVE INGREDIENT

Reference:	Grade, R. (1993), Report on the growth inhibition test of CGA 237218 tech. to green algae (*Scenedesmus subspicatus*). Unpublished Report No.

KMA 8.2.3/01	938007, 16.08.1993
Guideline:	OECD Guideline 201, Paris 1984.
GLP:	Yes
Material and methods:	The study was conducted during the period 24.05.1993 to 06.08.1993, by CIBA-GEIGY Ltd., CH-4002 Basel, Switzerland. The test material used was CGA 237218 tech., conc. of a.i.: not stated; batch no.: not stated. The possible inhibitory effects of CGA 237218 tech. on the unicellular alga *Scenedesmus subspicatus* were tested at nominal concentrations of 1.23, 3.7, 11, 33 and 100 mg test substance/L. Each test concentration was tested in 3 replicates, the blank control in 6. After 72 hours inhibition was determined.
Micro-organism	Bta Technical grade material CGA 237218 (GC 91)
Test species:	*Scenedesmus subspicatus*
Number of test algae:	Each test concentration was tested in 3 replicates, the blank control in 6
Treatments:	Nominal concentrations of 1.23, 3.7, 11, 33 and 100 mg test substance/L
Duration:	72 hours
Test conditions:	Not specified
Deviations from guideline	None
Endpoint:	No significant effects were detected at any concentration. Therefore, the E_bC_{50} was estimated to be > 56.5 mg CGA 237218 tech./L (equivalent to 3.6 x 10^9 CFU/L) with a confidence limit of 95 %. The NOE_bC was determined to be 56.5 mg CGA 237218 tech./L.
Observations:	The E_bC_{50} was estimated to be > 56.5 mg CGA 237218 tech./L with a confidence limit of 95 %. The NOE_bC was determined to be 56.5 mg CGA 237218 tech./L.

Results:

A summary of endpoints is given in the table below.

Table B.9.2.9.a: Toxicity effects/infectivity/pathogenicity of the MPCA to algae

Test species	*Scenedesmus subspicatus*
Toxicity	E_bC_{50}: > 3.6 x 10^9 CFU/L

	NOE_bC: 3.6 x 10^9 CFU/L
Infectivity / Pathogenicity	No signs of infectivity or pathogenicity found

Comments and conclusion RMS:

The study was previously evaluated in the DAR 2008 and considered acceptable. No effects were observed in this study. The 72-h E_bC_{50} from this study was determined to be 3.6 x 10^9 CFU/L and the 72-h NOEC to be 3.6 x 10^9 CFU/L. No signs of treatment related pathogenicity or infectivity were observed.

The OECD guideline 201 has been updated in 2006. As the test was performed in 1993 the previous version of the guideline from 1984 was used for the test. This is considered acceptable,

The main shortcomings of this study are listed in the following:

- Validity criteria (biomass increase in controls > factor 16 was not addressed).
- No reporting of origin and nature of TGAI,
- No reporting of culturing conditions and origin of test species
- No reporting of raw data (only area under the curve and calculated EC_{50} /NOEC values for biomass).
- No reporting of reference testing,
- No reporting of the detection method for algal biomass
- Initial biomass loading not reported

Considering that the test was performed under GLP and that the organism study is an entomopathogenic bacteria, it can be concluded that the study is reliable with restrictions.

Toxin/metabolite from microbial pest control agent (MPCA)

No study or information submitted. No study submitted.
The following information was included in the original version of the DAR:
"The Bta strain GC-91 does not produce metabolites toxic for human health or the environment. This is confirmed by the quality control tests during the fermentation and production process, respectively. The working vials will be tested for the amount of ß-exotoxins in the fermentation broth by HPLC analysis, fly test and mice injection. Furthermore, after the fermentation process the spores will be spray dried while the nutrient broth is discarded. If any metabolites would occur, they would be removed from the Technical Powder consequently (Chen & Hargrove, 2003)".

According to the information provided in Volume 1, it was demonstrated that Bta GC-91 can produce Cry1Ac, Cry1C, Cry1D and Cry2A insecticidal proteins. Apart from the Cry proteins several other insecticidal proteins produced by Bt and are contributing to their mode of action have been described as

well (vegetative insecticidal proteins VIP, cytolytic proteins Cyt etc.). Beta-exotoxins, are considered to have toxic properties but were shown not to be produced by commercial Bta strains.

Considering the type of application and the mode of action, the exposure of daphnids to metabolites of Bta GC-91 is not considered relevant.

B.9.2.10 Effects on plants other than algae

Bacillus thuringiensis spp. *aizawai* is toxic specifically to insects of the Lepidoptera order and no effects on aquatic plants from applications of Bta in insecticidal formulations targeted specifically at these insects is expected or envisaged. Furthermore, considering results from studies on algae and no reported negative effects from decades of use in agricultural and forestry environments, no further information is considered necessary.

New data 2016

No substantial new information is submitted for renewal of the strain according to Regulation (EC) No 1107/2009.

The literature search covering the last 10 years and focussing to target possible toxicity or pathogenicity of Bta on terrestrial plants did not provide any relevant information.

Products based on Bta GC-91 are used in Europe since several years without any report on adverse effects in treated plants. In addition, not any symptom of phytotoxicity has been noted during extensive efficacy testing of these products. Based on available experience with products based on Bta GC-91 and available knowledge about *Bacillus thuringiensis* subsp. *aizawai* in general it can be concluded that Bta GC-91 is not toxic, pathogenic or infective to terrestrial plants.

B.9.2.11 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
TOXICITY					
Rainbow trout (*Onchorhyncus mykiss*)	32 d	3.90 x 10^{10} CFU/L, 3.90 x 10^{9} CFU/L and 1.0 x 10^{9} CFU/L.	LC_{50} > 2.0 x 10^{10} CFU/L (mean measured concentration).	No signs of toxicity, pathogenicity or infectivity. Increased concentration of cfu were measured in the gills after	[redacted] [redacted](1991a)

Species	Test duration	Dose range	Results/ Endpoint	Observations	Reference
				necropsy, but did not contstitute infection	
Sheepshead minnow (*Cyprinodon variegatus*)	30 d	3.90 x 10^{10} CFU/L, 3.90 x 10^{9} CFU/L and 1.0 x 10^{9} CFU/L.	LC_{50} > 2.0 x 10^{10} CFU/L (mean measured concentration)	No signs of pathogenicity or infectivity.	[redacted] (1991b)
Daphnia magna	10 d	Not applicable	Not applicable	No endpoint calculated, due to different purpose of study. It concluded that heat labile components from the fermentation broth are carreied over into the technical material. These components elicit the toxicity of GC-91 observed for Daphnids.	Collins, M.K. (1997)
Daphnia magna	21 d	1.17 x 10^{10}, 3.51 x 10^{9}, 1.05 x 10^{9}, 3.16 x 10^{8}, 9.50 x 10^{7} CFU/L	21-day EC_{50} (survival): 3.24 x 10^{8} CFU/L. NOEC(length, offspring and survival): 1.57 x 10^{8} CFU/L	Signs of infectivity were not observed when appying the normal test procedures.	Christensen, K.P. (1991c)
Daphnia magna	21 d	8.5 x 10^{6}, 2.8 x	21-day EC_{50} >	No effects on	Collins, M.K.

Species	Test duration	Dose range	Results/ Endpoint	Observations	Reference
		10^7, 9.5 x 10^7, 3.15 x 10^8 and 1.05 x 10^9 CFU/L	6.2 x 10^8 CFU/L NOEC ≥ 6.2 x 10^8 CFU/L	pathogenicity or infectivity observed	(1993)
Palaemonetes vulgaris	30 d	1.58 x 10^{10} CFU/g	21-d NOEC 1.9 x 10^9 CFU/g (diet exposure)	No signs of treatment related pathogenicity or infectivity were observed.	Christensen, K.P. (1991d)
Scenedesmus subspicatus	72 h	4.2 X 10^7 14.1 X 10^7 46.5 X 10^7 125.5 X 10^7 356 X 10^7 CFU/L	E_bC_{50}: > 3.6 x 10^9 CFU/L NOE_bC: 3.6 x 10^9 CFU/L	No signs of infectivity or pathogenicity found	Grade, R. (1993)

B.9.3 Effects on Bees

B.9.3.1 Toxicity to bees

ACTIVE INGREDIENT

Reference: **KMA 8.3/01**	Winter, P.A. (1991a), CGA-237218: A dietary pathogenicity and toxicity study with the honey bee. Unpublished Report No. 108-310A, 05.07.1991
Guideline:	EPA Guideline No. 154°-24
GLP:	Yes
Material and methods:	The study was conducted during the period 22.05.1990 to 27.05.1990, by Wildlife International LTD., Maryland 21601, USA. The test material used was CGA 237218 tech., content of a.i.: 1.06 x 10^{11} spores/g; batch no.: P 903001. Honey bees (1 to 5 days of age at initiation of study) were exposed to nominal concentrations of 10^5, 10^7, and 10^8 CFU/g of feed. The attenuated control concentration was equal to the highest test concentration administered to the bees. Two replicates were assigned to each of the treatment and control groups by random draw. Each treatment and control replicate

	contained 25 individuals. Negative control bees were treated identically to all other bees, but did not receive the viable or attenuated test substance. Fresh diet was presented to the honey bees weekly. Photoperiod was 8 hours of light per day. Test temperatures at the time of observations ranged from 21°C to 25°C. Mean relative humidity was 61%. Observations on mortality and signs of toxicity were made twice on the day of initiation, then daily until termination of the study.
Micro-organism	Bta Technical grade material CGA 237218 (GC 91)
Test species:	Honey bee
Number of test animals:	Two replicates were assigned to each of the treatment and control groups by random draw. Each treatment and control replicate contained 25 individuals
Treatments:	Honey bees (1 to 5 days of age at initiation of study) were exposed to nominal concentrations of 10^5, 10^7, and 10^8 CFU/g of feed. The attenuated control concentration was equal to the highest test concentration administered to the bees. Negative control bees were treated identically to all other bees, but did not receive the viable or attenuated test substance.
Duration:	5 days
Test conditions:	Photoperiod was 8 hours of light per day. Test temperatures at the time of observations ranged from 21°C to 25°C. Mean relative humidity was 61%.
Deviations from guideline	Photoperiod of 8h light (versus total darkness)
Endpoint:	The dietary LC_{50} value for honey bees exposed to CGA-237218 was approximately 3 x 10^7 CFU/g with 95% confidence limits of 10^7 and 10^9 CFU/g. There may have been a slight increase in treatment related mortality in the 10^7 CFU/g treatment group
Observations:	Mortality in the negative and attenuated control reached 22% and 34%, respectively on Day 5. Immobile bees were observed in the negative control group on day 0

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	Honey bee
Toxicity	LC50 (oral): 3 x 10^7 CFU/g

Infectivity / Pathogenicity	Toxicity/ pathogenicity observed at 10^8 CFU/g. Infectivity not tested.

Comments and conclusion RMS:

The study was previously evaluated in the DAR 2008. The study was considered not sufficient to address the data requirement due to the high background mortality seen in the study. The previous conclusion was maintained and the study results cannot be used in the risk assessment.

Reference: **KMA 8.3/02**	Parrish, J.R. & Yeager, B. (1994), Honey bee toxicity feeding test/chronic - CGA-237218. Unpublished Report No. HB419, 30.06.1994
Guideline:	EPA Guideline No. 154°-24
GLP:	Yes
Material and methods:	The study was conducted during the period 12.07.1993 to 02.09.1993, by BIO/WEST, INC., Utah 84321, USA. CGA 237218 tech. (content of a.i.:not stated; batch no.: PI102002) was fed to adult worker honey bees (approx. 10-20 days old) at concentrations of 12800 ppm, 3200 ppm, 800 ppm, 200 ppm, 50 ppm, and 125 ppm in a 1:1/v:v/distilled water-honey feeding solution. A 12800 ppm attenuated control solution and a negative (non-attenuated) control feeding solution were also included in the test. Approximately 280 bees from each colony were treated (approximately 35 bees/solution/colony). Each feeding solution was administered ad libitum to the bees for the duration of 14 days. Each cage was placed in a temperature controlled and humidity monitored recovery room and maintained on a 12-hour on/12-hour off light cycle using artificial lighting. Observations to record general behaviour and mortality were completed approximately at 24 hour intervals following the initial feeding.
Micro-organism	Bta Technical grade material CGA 237218 (GC 91), 9.9% delta endotoxin,
Test species:	Honey bees
Number of test animals:	280 bees
Treatments:	Concentrations of 12800 ppm, 3200 ppm, 800 ppm, 200 ppm, 50 ppm, and 12.5 ppm in a 1:1/v:v/distilled water-honey feeding solution were tested; a 12800 ppm attenuated control solution and a negative (non-attenuated) control feeding solution were also included in the test

Duration:	14 days
Test conditions:	Temperature controlled and humidity monitored recovery room and maintained on a 12-hour on/12-hour off light cycle using artificial lighting
Deviations from guideline	None
Endpoint:	Consumption of feeding solutions which contained the active test substance displayed symptoms of poisoning, or at least intestinal distress, at the upper concentrations (i.e. 200 ppm – 12800 ppm). Cumulative mortality in the negative controls exceeded 1% on day 5, whereas cumulative mortalities in the lower test concentrations (i.e. 12.5 ppm, 50 ppm, and 200 ppm) remained below 1% until day 6 or 7. Cumulative mortalities in the negative controls exceeded 25% on day 11 of the study. Cumulative mortalities in the attenuated controls and the 12800 ppm study group were very similar, and total mortality was reached in both groups on day 7.
Observations:	Based on the statistical testing, the NOEC and LOEC values for day 5 were 800 ppm and 3200 ppm respectively, and for day 10 were 200 ppm and 800 ppm, respectively. However, although not statistically significant it was suggested in the report that the cumulative mortality in the 200 ppm treatment group on day 10 may be biologically significant and thus the final NOEC is set at 50 ppm. An LC_{50} of 3656 ppm was calculated for results obtained on day 5 of the study, and an LC_{50} of 170 ppm was calculated for day 10. The 5-day LD_{50} for technical CGA-237218 was 897 µg/bee and the 10-day LD_{50} was 91 µg/bee.

Results:

A summary of endpoints is given in the table below.

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

Test species	Honey bee
Toxicity	5-day LC_{50}: 3656 ppm (3652 mg/L, 897 µg/bee) 10-day LC_{50}: 170 ppm (169.8 mg/L, 91 µg/bee) NOEC: 50 ppm Delta-endotoxin (9.9%): 5-day LC_{50}: 88.8 µg/bee 10-dayLC_{50}: 9.0 µg/bee

Infectivity / Pathogenicity	Initial effects were very similar to insecticides causing stomach poisoning.

Comments and conclusion RMS:

The study was evaluated in the DAR from 2008 and was not considered acceptable as every second page was missing from the report, and therefore no reliable evaluation could be performed. The applicant resubmitted the study for the renewal of the active substance. The RMS re-evaluated the study based on this newly submitted study report.

The relevant guideline for this study is the OPPTS 885.4380 (1996) guideline, however also the OECD 245 (chronic oral toxicity test) has been considered for the re-evaluation.

Some deviations from the relevant guidelines were observed (i.e. age of bees, chilling, study duration, photoperiod, humidity). Also, no characterization of the test item in terms of CFU is included in the report and therefore results cannot be expressed in CFU/g. Considering that the control mortality exceeded 25% by day 11, it is considered acceptable to base the results on the mortality occurring through day 5 and day 10, as presented in the study report. Based on the delta-endotoxin content of the technical material, the results are expressed also in term of delta-endotoxin per bee. The test is valid and the results can be used for risk assessment.

Toxin/metabolite from microbial pest control agent (MPCA)

The Bta strain GC-91 does not produce metabolites toxic for human health or the environment. This is confirmed by the quality control tests during the fermentation and production process, respectively. The working vials will be tested for the amount of ß-exotoxins in the fermentation broth by HPLC analysis, fly test and mice injection. Furthermore, after the fermentation process the spores will be spray dried while the nutrient broth is discarded. If any metabolites would occur, they would be removed from the Technical Powder consequently (Chen & Hargrove, 2003).

New data 2016

Reference: Mommaerts, V., Jans, K., Smagghe, G. (2009). Impact of *Bacillus thuringiensis* strains on survival, reproduction and foraging behaviour in bumblebees (*Bombus terrestris*).
KMA 8.3/03 Pest Management Science, 66, 520-525

Guideline: Not specified

GLP: No

Material and methods: The objectives of the present researchwere to evaluate the side effects of

two Bt products, Dipel (Btk) and Xentari (Bta), on the bumblebee *Bombus terrestris* (L.). Workers were exposed in microcolonies of each 5 worker bees in the laboratory to the Bt products at their respective field recommended concentration through dermal contact and orally via feeding treated sugar water and pollen. For the contact applications, 50 µL of the aqueous concentration was topically applied on the dorsal thorax of each worker with a micropipette. For the oral treatments, the nests were exposed for 11 weeks to 500 mL of sugar water with the product or to pollen saturated the prepared recommended field rate concentration.
Observations of dead worker bees, male offspring in nests was done on a weekly basis.
The impact on foraging behaviour was tested as well by connecting two artificial nest boxes with a tube. In one box, five newly emerged workers constructed their nest. When third- and fourth-instar larvae appeared in the nest; food was removed from this box and placed in the second box.

Micro-organism *Bacillus thuringiensis* subsp. *aizawai* (in Xentari) and *Bacillus thuringiensis* subsp. *kurstaki* (in Dipel)

Test species: *Bombus terrestris*

Number of test animals: 20 worker bumble bees per treatment, in total 100 bumble bees (excluding the experiment on foraging behaviour)

Treatments: Microconlonies with 5 workers each, in replicates of 4 for each treatment route, 3 treatment routes: dermal contact, oral via treated sugar water, oral via treated pollen. 1 negative control (only water) and 1 positive control (Confidor, immidachloprid at recommended field concentration (0.1% in water).
Experiment performed twice independently.

Treatment was the recommended field rate of the product (0.1%) and a 1/10 dilution of the recommended field rate for Xentari (0.01%) in sugar water was additional tested tested due to the high mortality in the 0.1% treatment. Xentari WG contained 1.5 $x10^4$ IU/mg product and its recommended field rate was 0.1% formulation in water, leading to a recommended field rate of 1.5 x 10^4 IU/mL. Dipel contained 1.6 $x10^4$ IU/mg product and its recommended field rate was 0.1% formulation in water, leading to a recommended field rate of 1.6 x 10^4 IU/mL.

Duration: 11 weeks

Test conditions: 28-30°C, 60-70% humidity, continuous darkness

Deviations from guideline Not applicable.

Endpoint: Survival: number of surviving worker bees,
Reproduction: number of dead larvae and number of drones
Forageing bahaviour: effects on reproduction and larvae survival were measured

Observations: Xentari at its recommended field rate of 1.5 x10^4 IU/mL or 0.1% resulted in 100% acute worker mortality when delivered orally via treated sugar water. An additional test with a lower dose of 0.01% (1/10 of the MFRC) resulted in 0% mortality of the treated worker bees. Exposure to treated pollen at 0.1% did not lead to an increased mortality of workers.
Sublethal effects (loss of reproduction) of 100% were observed for the recommended field rate treatment (0.1%) when delivered via sugar water, while delivery via pollen resulted in 31% reduction in reproduction. Dermal contact exposure did not lead to a reduction in reproductive capability. No negative effects on reproduction with the lower dose (0.01%) were indicated, as there were no differences between the mean number of drones in the treated and control group after 11 weeks.
In the foraging behaviour test, no negative effects on microcolony performance were observed.
Dipel at its recommended field rate of 1.6 x10^4 IU/mL or 0.1% resulted in no mortality via contact exposure or when delivered orally via treated sugar water and treated pollen. No sublethal effects on reproduction were noted via oral treatment with Dipel via sugar water and pollen. In the foraging behaviour test, no negative effects were observed via drinking of contaminated water.
Conclusions: The results of exposing Dipel and Xentari to microcolonies of *B. terrestris* demonstrated that, in general, the Bt strains are safe to in this bumblebee, but in some cases there were detrimental effects that depend on strain and route of exposure. The authors state that routine testing of lethal and sublethal effects is recommended to ascertain a safe combined use of Bt products and bumblebees in modern agriculture.

Results:

A summary of endpoints is given in the table below.

Table B.9.3.1.c: Toxic effects of the MPCA to bees

Test species	*Bombus terrestris* (Bumble bee)
Toxicity	Xentari: 100% mortality orally via sugar water at 1.5 x 10^4 IU/mL 0% mortality orally via sugar water at 1.5 x 10^3 IU/mL 100% reduction in reproduction orally via sugar water at 1.5 x 10^4 IU/mL 0% reduction in reproduction orally via sugar water at 1.5 x 10^3 IU/mL 31% reduction in reproduction orally via pollen at 1.5 x 10^4 IU/mL Dipel: 3% mortality via contact at 1.6 x10^4 IU/mL 5% mortality via sugar water at 1.6 x10^4 IU/mL 0% mortality via treated pollen at 1.6 x10^4 IU/mL No effects on reproduction at 1.6 x10^4 IU/mL
Infectivity / Pathogenicity	Not tested.

Comments and conclusion RMS:

This scientific paper did not apply the standards of any guideline or GLP. While the quality of the scientific study itself is acceptable and provides valuable supporting information, the outcome of the study itself is difficult to translate into appropriate use for regulatory purposes. While the used product have been identified through the trade names, the exact strains, the manufacturers, the source and the Lot or Batch numbers were not reported. Equally the activity of the product was only reported in IU/mg formulated product which refers to the activity in terms of Bt toxin.

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

No effects on bumble bees were seen for the Btk strain present in Dipel.

The difference in the effects between the two products can be explained by the presence of different Cry proteins present in the two strains, 1Aa, 1Ab, 1Ac, 2Aa, 2Ab, 1 la in Dipel and 1Ab, 1C and 1D in Xentari. According to the study authors, "*it is of great interest to know how the active Cry toxins achieved their insecticidal effects in the bumblebee workers. The toxic mechanism of Cry toxins is based on the presence of sensitive receptor(s) in the brush border membrane of the insect midgut, provoking an altered gut physiology with decreased proteinase activities and epithelium lesions. Im-*

pairment in the insect midgut structure and function can then lead to starvation and finally death of the treated insect. Thus, Cry toxin(s) activity in the Hymenoptera may indicate specific and new binding receptors/sites".

It is unknown whether the Bta and Btk strains used in this study contain the same plasmids as the two strains used for the production of the transconjugant strain Bta GC-91. As a result, the conclusion from this study is that there can be effects on bumblebees however, it is uncertain what the real effect of the transconjugant will be.

The study is acceptable and the results can be used in the risk assessment in a weight-of-evidence.

Reference	Report: del Mar Leza, M., Llado, G., Petro, A.B., Alemany, A. (2014)
KMA 8.3/04	First field assessment of *Bacillus thuringiensis* subsp. *kurstak*i aerial application on the colony performance of *Apis mellifera* L. (Hymenoptera: Apidae) Spanish Journal of Agricultural Research, 12(2), 405-408
Guideline:	Not specified
GLP:	No
Materials and Methods	The aim of this study was to analyse the effect of field aerial applications of Btk on bee colony performance, specifically on the brood cell percentage evolution, which can be used as an indicator of queen health and brood development breeding rates. The field study was conducted in Spain. In order to confirm or reject whether Btk aerial treatment affect the colony performance of honeybees in field conditions, the assay analyses the evolution of the percentage of each frame occupied with brood. Eight Langstroth nucleus hives were located in two pine forests of Ibiza, West Mediterranean island. One forest was located in a zone treated with Btk, while the second was in a treatment-free protected area (control). A Before-After Control-Impact design was conducted in the study. The first pictures were taken before the treatment was applied, the first of five samplings after the treatment were taken fortnightly, except the last sampling (1 month later). Both faces of every frame were photographed in every sampling. Each digital photograph was processed with the Image Analysis Software SIG Arc GIS (ESRI) in order to calculate the percentage of cells occupied with brood in relation to the total surface of the frame, as an effective measurement of the bee's brooding efficiency. Data were analysed by a 2x (6 x 4) mixed factorial design with one between-factor (control/treated) and one within-factor (six temporal points) in SPSS v 20.0 at a significance level of $p<0.05$.
Micro-organism	*Bacillus thuringiensis* subsp. *kurstakii*

Test species:	*Apis mellifera* (Honey bee)
Number of test animals:	8 hives (colonies) distributed over 2 sites one control and one treatment sites (4 assigned each to treatment and control area)
Treatments:	Aerial (helicopter) spray of Foray 48 B SC at 11.8% p/v, 11.8 x 10^6 IU/g at 3.5 L/ha and a drop diameter of 100-125 microns on pine forest
Duration:	August 25 2009 to December 16 2009 (spray was in October 2009)
Test conditions:	Field study, natural field conditions, not reported
Deviations from guideline	Not applicable
Endpoint:	Pictures of frames were taken at the beginning and 6 later time points and analysed with imaging software to calculate the percentage of cells occupied with brood (open or capped) in relation to the total surface area of the frame (bee brooding efficiency) in time
Observations:	The percentage of brood in both groups of hives showed a strong parallelism throughout the experiment. No significant differences between groups were found. During the first three samplings the broods increased. In the fourth sampling (after the treatment with Btk) three colonies of the treated site and all colonies of the control site the brood began to decrease. In the fifth sampling, the brood surface was practically non-existent in three hives of the treated colonies and three hives of the control. In addition, new queen cells in all hives were observed, as well as new honeybee swarms in the nearby trees. All of these symptoms suggest that the hives had lost their queens because of natural swarming process. However, when comparing the brood percentage of both groups through the ratio efficiency Btk/control, it can be observed that even though the Btk hives had an initial brood surface smaller than those of the control group, the brood mean ratio increased throughout the experiment. Therefore, the results of the present study suggest that Btk aerial applications did not affect the brood development of honeybees under natural conditions. Nevertheless further field studies are required to ascertain a safe use of Btk in forest pest management.

Results:

A summary of endpoints is given in the table below.

Table B.9.3.1.d: Toxic effects of the MPCA to bees

Test species	*Apis mellifera* (Honey bee)
Toxicity	No significant difference was seen in the development of broods when comparing the treated pine tree site (3.5 L product/ha) with the untreated pine tree site. The breakdown of broods after application was attributed to the natural swarming process of bees.
Infectivity / Pathogenicity	Not tested.

Comments and conclusion RMS:

The study aimed at investigating potential effects of Btk (which is not the same species as the one under evaluation) on bee colony performance and colony health under natural field conditions and in relation to aerial applications.

While the used product has been identified through the trade name and supplier, the exact strains, and the Lot or Batch numbers were not reported. The results are presented as International Units (IU) which refers to the activity in terms of Bt toxin.

Furthermore, the paper does not report on the origin of the bees and the history of the queens used to establish the nuclei hives. Also, it is not clear, if the untreated areas and hives could have been other-wise disturbed or exposed to adverse agents.

This study may indicate that aerial applications of Btk at the specific rate do not have adverse effects on bee colony health, reproduction and natural swarming events, but as the paper states itself further research is necessary to confirm the findings. For regulatory purposes no direct use is possible.

The information provided can be regarded as supplementary.

B.9.3.2 Infectiveness to bees

No information provided.

B.9.3.3 Pathogenicity to bees

No information provided.

B.9.3.4 Effects on arthropods other than bees (Annex IIM 8.4; Annex IIIB 10.4)

B.9.3.5 Toxicity to arthropods other than bees

Reference: **KMA 8.4/01**	Thompson, M.M., Hoxter, K.A., Smith, G.J. (1991): CGA-237218: A dietary pathogenicity and toxicity study with the green lacewing larvae. Unpublished Report No. 108-312, 05.07.1991
Guideline:	FIFRA Guideline 154A-23
GLP:	Yes
Material and methods:	The study was conducted during the period 16.11.1989 to 30.11.1987, by Ciba-Geigy Corporation, Greensboro, NC 27419, USA. The test material used was CGA-237218 (conc. of a.i.: 1.06 x 10^{11} CFU/g, batch code: P903001). The green lacewing larvae *Chrysopa carnea* was exposed to CGA-237218 in the diet for 5 days followed by a 9-day post exposure period. Three treatment levels, 10^5, 10^7, and 10^9 colony forming units per gram of feed, were tested along with an attenuated control and a negative control. The attenuated control concentration was equal to the highest test concentration administered to the larvae. Negative control larvae were treated identically to all other larvae, but did not receive the viable or attenuated test substance. Thirty replicates were assigned to each of the treatment and control groups by random draw. Each treatment and control replicate contained one individual. The larvae were fed the pollen substitute for five days, then were fed untreated eggs of the Angoumois grain moth for the remainder of the study. The photoperiod was 8 hours of light per day. Test temperatures ranged from 22°C to 24°C. The average relative humidity was 46%. Observations on mortality and signs of toxicity were made twice on the day of test initiation and then once each day until study termination.
Micro-organism	Bta Technical grade material CGA 237218 (GC 91)
Test species:	*Chrysopa carnea* (green lacewing)
Number of test animals:	Thirty replicates were assigned to each of the treatment and control groups by random draw. Each treatment and control replicate contained one individual. 150 test animals.
Treatments:	Three treatment levels, 10^5, 10^7, and 10^9 colony forming units per gram of feed, were tested along with an attenuated control and a negative control.
Duration:	14 days (of which 5 days of exposure)
Test conditions:	The photoperiod was 8 hours of light per day. Test temperatures ranged

	from 22°C to 24°C. The average relative humidity was 46%.
Deviations from guideline	None
Endpoint:	At study termination, total mortality in the negative control and attenuated control and attenuated control was 23% and 37%, respectively. No clinical signs of toxicity were observed in these groups. There was an increase in mortality for the attenuated control in comparison to the negative control, but this appeared to be incidental. The study was terminated at the end of 14 days when the mortality in the negative control exceeded 20%. Mortalities at study termination in the 10^5, 10^7 and 10^9 CFU/g feed concentrations were 20%, 3% and 33%, respectively. The pattern of mortality did not appear to be dose responsive or treatment related. No overt signs of toxicity were noted in these groups.
Observations:	The LC_{50} value for the green lacewing *Chrysopa carnea* was determined to be greater than 10^9 CFU/g feed, the highest concentration tested. The no-observed-effect level was 10^9 CFU/g feed.

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	*Chysopa carnea* (green lacewing)
Toxicity	LD_{50}: > 10^9 CFU/g feed
Infectivity / Pathogenicity	No signs of infectivity or pathogenicity observed. Infectivity not tested.

Comments and conclusion RMS:

The study was previously evaluated in the DAR from 2008. Due to the high control mortalities further data were recommended.

The study was re-evaluated by the RMS and compared to the current applicable guidelines. For the study the predecessor of the currently applicable OPPTS 885.340 (1996) was used.

Due to the high mortality in the control, the data cannot be used in the risk assessment. The conclusion from the DAR (2008) is maintained and the study is not considered acceptable for use in risk assessment.

Reference: **KMA 8.4/02**	Winter, P.A., Hoxter, K.A., Smith, G.J. (1991b): CGA-237218: A dietary and toxicity study with the parasitic Hymenopteran *Uga menoni*. Unpublished Report No. 108-311A, 05.07.1991
Guideline:	FIFRA Guideline 154A-23
GLP:	None
Material and methods:	The study was conducted during the period 31.05.1990 to 30.06.1990, by Ciba-Geigy Corporation, Greensboro, NC 27419, USA. The test material used was CGA-237218 (conc. of a.i.: 1.06 x 10^{11} CFU/g, batch code: P903001). The parasitic hymenopteran *Uga menoni* was exposed to CGA-237218 in the diet for 30 days. Three treatment levels, 10^5, 10^7, and 10^9 colony forming units per gram of feed, were tested along with an attenuated control and a negative control. The attenuated control concentration was equal to the highest test concentration administered to the wasps. Negative control wasps were treated identically to all others, but did not receive the viable or attenuated test substance. Two replicates were assigned to each of the treatment and control groups by random draw. Each treatment and control replicate contained 25 individuals. The food source was available ad libitum to the insects. The photoperiod was 8 hours of light per day. Test temperatures ranged from 19°C to 24°C. The average relative humidity was 78%. Observations on mortality and signs of toxicity were made twice on the day of test initiation and then daily for 30 days.
Micro-organism	Bta Technical grade material CGA 237218 (GC 91)
Test species:	*Uga menoni*
Number of test animals:	Two replicates were assigned to each of the treatment and control groups by random draw. Each treatment and control replicate contained 25 individuals.
Treatments:	Three treatment levels, 10^5, 10^7, and 10^9 colony forming units per gram of feed, were tested along with an attenuated control and a negative control
Duration:	30 days.
Test conditions:	The photoperiod was 8 hours of light per day. Test temperatures ranged from 19°C to 24°C. The average relative humidity was 78%.
Deviations from guideline	None
Endpoint:	At test termination, total mortality in the negative control and attenuated control groups was 18% and 36%, respectively. Mortality at test termination in the 10^5 and 10^7 CFU/g feed concentrations was 20% and 18%, respectively. This mortality was comparable to the negative control group and was not

	attributed to treatment. Mortality at the 10^9 CFU/g feed concentration was 100%. An increase in the mortality rate was apparent in the second week of the study and resulted in total mortality by day 28. Mortality at that concentration was considered to be treatment related. Surviving insects were normal in appearance and behaviour throughout the test period in all treatment groups.
Observations:	The dietary LC_{50} value for the parasitic hymenopteran *Uga menoni* was approximately 4.4 x 10^7 CFU/ feed with a 95% confidence interval of 10^7 to 10^9 CFU/g feed. The no-observed-effect level was 10^7 CFU/g based on treatment related mortality at the highest test concentration.

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	*Uga menoni*
Toxicity	30 day dietary LC50: 4.4 x 10^7 CFU/g feed 30 day dietary NOEC: 10^7 CFU/g feed
Infectivity / Pathogenicity	Infectivity not tested.

Comments and conclusion RMS:

The study was previously evaluated in the DAR from 2008. From that evaluation is not clear if the control mortalities were considered too high for the study to be accepted.

The study was re-evaluated by the RMS and compared to the current applicable guidelines. For the study the predecessor of the currently applicable OPPTS 885.340 (1996) was used. No specific guideline is available for this test species.

The mortality in the attenuated control (36%) exceeded 20% by the end of the test, which suggests that endotoxins released from the cells during inactivation may have had an effect on the wasps. The control mortality in the negative control was 18%, which is just below 20. Considering the long duration of the study and the finding of similar mortalities in the two lower concentrations this is not considered problematic, as also according to OPPTS 885.340 higher control mortalities should only lead to an earlier termination.

The results produced in this study may therefore be used in risk assessment.

DAR-March 2008

Reference: **KMA 8.4/03**	Thompson, M.M., Hoxter, K.A., Jaber, M. (1991): CGA-237218: A dietary pathogenicity and toxicity study with ladybird beetles. Unpublished Report No. 108-313, 05.07.1991
Guideline:	EPA Guideline 154A-23
GLP:	Yes
Material and methods:	The study was conducted during the period 16.11.1989 to 21.11.1989, by Ciba-Geigy Corporation, Greensboro, NC 27419, USA. The test material used was CGA-237218 (conc. of a.i.: 1.06 x 10^{11} CFU/g, batch code: P903001). The ladybird beetle (*Hippodamia convergens*) was exposed to CGA-237218 in the diet for 5 days. Three treatment levels, 10^5, 10^7, and 10^9 colony forming units per gram of feed, were tested along with an attenuated control and a negative control. The attenuated control concentration was equal to the highest test concentration administered to the ladybird beetles. Negative control beetles were treated identically to all others, but did not receive the viable or attenuated test substance. Two replicates were assigned to each of the treatment and control groups by random draw. Each treatment and control replicate contained 25 individuals. The food source was available ad libitum to the insects. The photoperiod was 8 hours of light per day. Test temperatures ranged from 22°C to 24°C. The average relative humidity was 42%. Observations on mortality and signs of toxicity were made twice on the day of test initiation and then daily until termination of the study.
Test substance	Bta Technical grade material CGA 237218 (GC 91)
Test species:	The ladybird beetle (*Hippodamia convergens*)
Number of test animals:	Each treatment and control replicate contained 25 individuals, each treatment was replicated twice
Treatments:	Three treatment levels, 10^5, 10^7, and 10^9 colony forming units per gram of feed, were tested along with an attenuated control and a negative control
Duration:	5 days
Test conditions:	The photoperiod was 8 hours of light per day. Test temperatures ranged from 22°C to 24°C. The average relative humidity was 42%.
Deviations from guideline	None
Endpoint:	At study termination, total mortality in the negative control and attenuated control groups was 26% and 12%, respectively. The study was terminated when the negative control mortality exceeded 20%. Mortalities in the 10^5, 10^7 and 10^9 CFU/g feed concentrations were 4%, 6% and 8%, respectively and

	did not appear to be treatment related. No overt signs of toxicity were observed at any treatment group.
Observations:	The LC_{50} value for ladybird beetles exposed to CGA-237218 in the diet for five days was greater than 10^9 CFU/g feed.

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	5-day dietary LC_{50}: $>10^9$ CFU/g feed
Infectivity / Pathogenicity	No signs of pathogenicity observed. Infectivity not tested.

Comments and conclusion RMS:

The study was previously evaluated in the DAR from 2008. From that evaluation is not clear if the control mortalities were considered too high for the study to be accepted.

The study was re-evaluated by the RMS and compared to the current applicable guidelines. For the study the predecessor of the currently applicable OPPTS 885.340 (1996) was used. No specific guideline is available for this test species.

In accordance with the guideline, the study was terminated already after 5 days, because negative control mortality exceeded 20%. However, normally the study duration should be 30 days. As control mortalities increased very quickly the suitability of the test system is questionable. Within the 5 day period, a direct comparison to the treatments can be made, but it is questionable how useful and relevant this is for risk assessment.

New data 2016

Reference: Bernard, M.B., Cole, P., Kobelt, A., Horne, P.A., Altmann, J., Wratten, S.D., Yen, A.L. (2010)

KIIM 8.4/04 Reducing the impact of pesticides on biological control in Australian vineyards: Pesticide mortality and fecundity effects on an indicator species, the predatory mite *Euseius victoriensis* (Acari: Phytoseiidae)

Journal of Economic Entomology, 103(6), 2061-2071

Guideline: Not specified.

GLP:	No.
Material and methods:	Laboratory bioassays on detached soybean, *Glycine max* (L.) Merr. leaves were used to test 23 fungicides, five insecticides, two acaricides, one herbicide, and two adjuvants on a key Australian predatory mite species *Euseius victoriensis* (Womersley) in "worst-case scenario" direct overspray assays. The study was conducted in the laboratory. Zero- to 48-h-old juveniles, their initial food, and water supply were sprayed to runoff with a Potter tower. Cumulative mortality was assessed 48 h, 4 d, and 7 d after spraying. Fecundity was assessed for *Bacillus thuringiensis* subsp. kurstaki ed for 7 d from start of oviposition. Seven assays with 32 compounds were tested; one was a commercial microbial product of *Bacillus thuringiensis* subsp. *kurstaki* (Btk) (Delfin WG, 0.57g/L). Only results on Delfin WG are reported in this summary.
Micro-organism	*Bacillus thuringiensis* (Bt) subsp. *kurstaki*
Test species:	Predatory mite *Euseius victoriensis*
Number of test animals:	22-25 eggs where brought to hatching in test container, juveniles were counted and dead larvae removed, at maturity sex ratio was adjusted to ≥ 1 male: 2 females
Treatments:	4 replicates at a (worse case spray deposit at runoff point) occuring at the maximum application rate of 100g /100L spray solution (at 1000 L/ha), which equals 1kg product/ha.
Duration:	In total 12 days 7 days post-treatment to assess mortality up to 12 days post-treatment (to assess fecundity)
Test conditions:	Temperature 24.0 ± 1°C, Relative humidity 80 ± 10%, Photoperiod of 16:8 (L:D) h, and 750-1050 lux, in an externally vented constant temperature cabinet
Deviations from guideline	Not applicable
Endpoint:	Mortality was assessed 48h, 4d and 7d after spraying Fecundity was assessed at 5-12 days after spraying (larvae + eggs) Assessments were made under a dissecting microscope.
Observations:	Mortality at 7 days post application was at 3.41 %, which was not significantly different from the control.

Fecundity was also not negatively affected.

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	*Euseius victoriensis* (predatory mite)
Toxicity	No LC_{50} derived, however no effects at applied single concentration, therefore: Estiamted LC_{50}: > 1kg product/ha (a.s. content of Delfin WG unknown)
Infectivity / Pathogenicity	No signs of pathogenicity and or infectivity observed.

Comments and conclusion RMS:

This publication was submitted by the applicant for renewal of the active substance and has not previously been evaluated.

As this is a scientific publication no guidance and/or GLP standards were applied to the experimental work. The test substance is not clearly identified and amount of active ingredient and missing strain identification. Furthermore the tested species is *Bacillus thuringiensis* subsp *kurstakii.* It is also not known what the co-formulants and impurities in the tested formulation were. Due to these reasons the study was assigned reliable with restrictions.

Reference:

KIIM 8.4/05 **:** Carvalho, G.A., Moura, A.P., Bueno, V.H.P. (2006)

Side effects of pesticides on *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae)

Integrated Control in Protected Crops, Mediterranean Climate, IOBC/wprs Bulletin, 26(4), 355-359

Guideline: Not specified

GLP: No

Abstract One of the most promising biological agents for controlling the tomato moth *Tuta absoluta* under protected cultivation in Brazil is the egg parasitoid *Trichogramma pretiosum* (Hym., Trichogrammatidae). However, there is currently little knowledge of the toxicity of pesticides commonly used in tomato crops to this parasitoid. This work aimed to analyse the side-effects of 24 pesticides on *T. pretiosum*. These compounds are all commonly used to control tomato crop pests and diseases in Brazil. The pesticides assessed: Orthene (acephate), Mospilan (acetamiprid), Atabron (chlorfluazuron), Trigard (cyromazine), Forum (dimethomorph), Pi-rimor (pirimicarb), Mimic (tebufenozide), Nomolt (teflubenzuron), Actara (thiamethoxam), Alsystin (triflumuron), Dipel (*Bacillus thuringiensis*), Benlate (benomyl), Bravonil (chlorothalonil), Rovral (iprodione) and Dithane (mancozeb) were shown to be harmless to *T. pretiosum*. Calypso (thiacloprid), Confidor (imidacloprid), Cartap (cartap), Decis (deltamethrin), Karate (lambdacyhalothrin), Pirate (chlorfenapyr) and Tamaron (methamidophos) showed the highest toxicity to this parasitoid species. The pesticides belonging to the organophosphate and pyrethroid classes presented the greatest toxicities to *T. pretiosum*, whereas the neonicotinoids, insect growth regulators, fungicides and microbial *B. thuringiensis* tested proved harmless to *T. pretiosum*.

Materials and Methods: The study was conducted in the laboratory. Pesticides were tested on both the least and the most susceptible life stages of *T. pretiosum*. The effects of the different compounds were investigated in order to assess the effects of pesticides on the parasitization capacity and emergence success of the parasitoid. The pesticides were classified in four toxicological categories: class 1 = harmless (< 30% reduction), class 2 = slightly harmful (30 - 79%), class 3 = moderately harmful (80 - 99%) and class 4 = harmful (> 99%).

Other experiments of the study are not presented here.

Findings & Conclusion: *Bacillus thuringiensis* subsp. *kurstaki* was harmless to *T. pretiosum* in the laboratory tests.

Comments and conclusion RMS:

According to the study authors Btk was harmless to *T. pretiosum* and therefore can be recommended for the use in IPM programs. The information can be regarded as supplementary.

Reference:

KMA 8.4/06: Garcia, P.V., Pereira, N., Oliveira, L.M. (2009)

Side-effects of organic and synthetic pesticides on cold-stored diapausing prepupae of *Trichogramma cordubensis*

BioControl, 54, 451-458

Guideline: Not specified

GLP: No

Abstract The side-effects of three insecticides (deltamethrin, lambda-cyhalothrin and *Bacillus thuringiensis* Berliner) and one fungicide (basic copper sulphate) were tested on cold-stored diapausing prepupae of *Trichogramma cordubensis* Vargas and Cabello (Hymenoptera: Trichogrammatidae). Pesticides were directly sprayed on parasitized host eggs (being the diapausing parasitoids in the prepupal stage) after cold storage (3°C) for three different periods (60, 120 and 180 days). Regardless of the period of cold storage, both pyrethroids reduced the emergence rates of *T. cordubensis* (both < 25%) compared to the control (emergence varied from 83% to 89%). The most toxic pyrethroid was lambda-cyhalothrin; *Trichogramma cordubensis* adult emergence varied from 7% to 15%. Lambda-cyhalothrin also negatively affected the longevity and fecundity of parasitoids cold stored for 60 days. *Bacillus thuringiensis* Berliner subsp. *kurstaki* and basic copper sulphate had little or no adverse effect on emergence rates (generally > 80%), longevity nor fecundity of *T. cordubensis*, indicating that these pesticides could be successfully integrated into pest management programs using wasps that were cold stored under diapause. Such integration would be valuable to pest management programs by reducing the costs of *T. cordubensis* mass rearing and by allowing producers to stockpile parasitoids for release in the growing season. However, since the emergence rate, longevity and fecundity of *T. cordubensis* generally decreased with increasing duration in cold storage, wasps to be used in integrated pest management programs should only be stored at 3°C for 60 days maximum.

Materials and Methods: The study was conducted in the laboratory. Diapause of *T. cordubensis* was induced during the pre-imaginal development. Groups comprising five egg cards (with parasitized host eggs, being the diapausing parasitoids in the prepupal stage) were then sprayed with the aqueous suspension of the pesticide or distilled water (control). Adult emergence rate was estimated per egg card by dividing the number of blackened host eggs (i.e. parasitized eggs) with emergence holes by the total number of blackened eggs. The emerged parasitoids were then used in experiments to evaluate their longevity and fecundity. The number of dead females was checked daily, fecundity was determined by counting the number of parasitized host eggs that turned black. Other experiments of the study are not presented here.

Findings: Adult emergence rate of wasps treated with *B. thuringiensis* was > 80 ± 0.02% and

was similar to the control treatments. Parasitoids treated with *B. thuringiensis* had similar longevities compared to the control treatment. A decrease in emergence rate was observed with increase in duration of cold storage, being generally lower for parasitoids cold stored for 180 days than 60 days. Longevity decreased with increase in duration of cold storage and was lower for parasitioids cold stored for 180 days than 120 days or 60 days. Fecundity of parasitoids treated with *B. thuringiensis* was generally close to the control treatments. Similarly to emergence rates and longevity, fecundity of the parasitoids decreased with increase of cold storage, being considerably lower after 120 and 180 days of storage than 60 days.

Conclusions: *Bacillus thuringiensis* had little or no adverse effect on emergence rates, longevity and fecundity of *T. cordubensis*, indicating that this pesticide could be successfully integrated into pest control programs using wasps that were cold-stored under diapausing pre-imaginal stages.

Comments and conclusion RMS:

This study demonstrated that *B.thuringiensis* had no effects on emergence rates, longevity and fecundity of *T. cordubensis* thereby demonstrating that it can be used in the IPM programs. The information can be regarded as supplementary.

Reference:

KIIM 8.4/07: Garantonakis, N., Varikou, K., Birouraki, A. (2016)

Comparative selectivity of pesticides used in greenhouses, on the aphid parasitoid *Aphidius colemani* (Hymenoptera: Braconidae)

Biocontrol Science and Technology, 5-6, 678-690

Guideline: Not specified

GLP: No

Abstract A series of bioassays were conducted under laboratory conditions to determine the relative toxicities of various pesticides (acetamiprid, cypermethrin, chlorantraniliprole and emamectin benzoate, *Bacillus thuringiensis* var. *kurstaki*, and *Helicoverpa armigera* nucleopolyhedrovirus, copper oxychloride, iprodione, mandipropamid, a mixture of propamocarb + fluopicolide and mixture of fludioxonil + cyperprodinil) on *Aphidius colemani* adults and mummies, as well as sublethal effects on female fecundity. Cypermethrin was highly toxic to pupa of *A. colemani* within host mummies. Acetamiprid, cypermethrin, emamectin benzoate, a mixture of propamocarb + fluopicolide and mixture of fludioxonil + cyprodinil were also highly toxic to *A. colemani* adults (92 - 100% mortality at 48 h post treatment). Mandipropamid, iprodione and copper oxychloride treatments significantly reduced fecundity of the female parasitoids. In contrast, *B. thuringiensis* var. *kurstaki*, *H. armigera* nucleopolyhedrovirus

and chlorantraniliprole were harmless (< 30% mortality) to the parasitoid species tested according to International Organisation for Biological Control toxicity classification and are likely to be compatible with integrated pest management programmes.

Materials and Methods: The study was conducted in the laboratory. All the pesticides were diluted in water and tested at the highest recommended field rates according to their label guidelines compared to a control (distilled water). To evaluate the contact toxicities of pesticides against mummies, 100 pupae (24 - 48 h old) developing in *Rhopalosiphum padi* per treatment were sprayed at the rate of 2 ± 0.2 mg/cm^2. After application, mummies were removed and the effect was evaluated after 10 days by counting the number of *A. colemani* adults that emerged. For the evaluation of contact toxicity for the adults of *A. colemani*, two glass plates were sprayed at the rate of 2 ± 0.2 mg/cm^2. After treated glass plates dried for 1 h, < 24 h old female parasitoids (40 per treatment) were introduced into the test unit. Mortality was recorded after 2, 24 and 48 h. The surviving females were collected and used in the sublethal effects assessment. To evaluate the sublethal effects on female fecundity, the surviving females were introduced individually to potted barley infested with approx. 100 *R. padi* nymphs (15 replicates per treatment). After 24 h, the female wasps were removed and the number of mummified aphids produced per female was counted after 14 days.

Findings: *Bacillus thuringiensis* var. *kurstaki* was classified as harmless for the contact toxicity against mummies (86% of *A. colemani* successfully emerging form mummies). The contact toxicity to adults of *A. colemani* was harmless and caused 14% mortality. A 27% reduction of fecundity was observed, according to the IOBC TC, *Bacillus thuringiensis* var. *kurstaki* was classified as harmless.

Other findings of the study are not presented here.

Conclusions: *Bacillus thuringiensis* var. *kurstaki* was classified as harmless to *Aphidius colemani* according to the IOBC TC regarding emergence rate, adult mortality and fecundity.

Comments and conclusion RMS:

The test was conducted according to IOBC guidelines. However, no reference item was included. The mortality in the control did not exceed 13%. The mean number of recorded mummies per female was 16.73 after a 48 h exposure. As no raw data were presented it cannot be verified if there were no more than two wasps producing zero values. *Bacillus thuringiensis* var. *kurstaki* was classified as harmless to *A. colemani* when glass plates were sprayed with to 2 mg product/cm2 of 3% a.i. (i.e. 6 x 10^3 kg a.i./ha).

The study is considered reliable with restrictions.

Reference:

KIIM 8.4/08: Momanyi, G., Maranga, R., Sithanantham, S., Agong, S., Matoka, C.M., Hassan, S.A. (2012)

Evaluation of persistence and relative toxicity of some pest control products to adults of two native trichogrammatid species in Kenya

BioControl, 57, 591-601

Guideline: Not specified

GLP: No

Abstract The utilization of native trichogrammatids for biocontrol of *Helicoverpa armigera* (Hbn.) and their potential integration with pesticide use are currently receiving attention. In this study the interaction of adults of *Trichogramma* sp. nr. *mwanzai* and *Trichogramma* sp. nr. *lutea* with commonly used pesticide products was investigated. The toxicity of eight pest control products commonly used in vegetable corps in Kenya, were evaluated by exposing the adults of the two species to detached potted tomato leaves at different intervals after spraying. Two biologically derived products, Achook® (neem-based) and Dipel® (*Bt* ssp. *kurstaki*) - were found to be harmless and had no persistent toxicity on both the trichogrammatid species. Two organophosphate products tested, dimethoate (Rogor®) and malathion (Malathion®) were found to be 'slightly harmful' and 'moderately persistent' respectively. Three other synthetic insecticides, lambdacyhalothrin (Karate®), bifenthrin (Brigade®) and alpha-cypermethrin (Fastac®) were found to be 'moderately harmful' and 'persistent' respectively. On the basis of five concentrations tested (0.032, 0.016, 0.008, 0.004 and 0.002 of the recommended field rates) the LC_{50} values for adult *T. sp. nr. mwanzai* were estimated as 285, 411, 435 and 1.916 (g active ingredient (a.i.) mL^{-1}) for dimethoate, malathion, lambdacyhalothrin as well as cypermethrin + profenofos respectively. The corresponding values for *T. sp. nr. lutea* were 247, 251, 278 and 697 respectively. Further, Karate® appeared to be the least toxic among the four products, across all the respective concentrations. The study was an attempt to adopt the methodologies of the IOBC (International Organisation for Biological Control) on non-target risk assessment for pest control products to cater for the local needs of integrating the use of the trichogrammatids along with other pest control products.

Materials and Methods: The study was conducted in the laboratory. For evaluation of residual toxicity, *Bt* ssp. *kurstaki* (Dipel ® 32000 IU/mg; 1 g/L) was sprayed on one month od green tomato plants. Sprayed leaves were removed at post-spray intervals of 2, 5, 10, 15, 20, 25, 30 and 35 days. Twenty female adults of *Trichogramma* sp. nr. *mwanzai* and *Trichogramma* sp. nr. *lutea* were exposed to the treated leaves; 150 host eggs of *Corcyra cephalonica* were offered to oviposit in the test unit. The number of eggs parasitised and the progeny adults that emerged from the parasitized were recorded. For evaluation of the toxicity on trichogrammatid immature stages, 150 host eggs of *C. cephalonica* were offered to twenty female adults of *T.* sp. nr. *mwanzai* and *T.* sp. nr. *lutea* for oviposition. After 24 h, the adults were removed and the parasitized host eggs were then sprayed on days 1, 2, 3, 4, 5, 6, 7, 8 and 9. The number of eggs parasitized (eggs that turned black) as well as the

number of adults emerged were recorded.

Other experiments of the study are not presented here.

Findings: The parasitization was slightly reduced by Dipel® on day 5 and 10 compared to the control. However, no difference in parasitization was observed on days 15 and 20 for both trichogrammatids. There were no adverse effects on the pre-imaginal development of *T.* sp. nr. *mwanzai* and *T.* sp. nr. *lutea* between day 1 (egg-larval stage) and day 3 (pre-pupal stage); both were considered to be safe for the pre-imaginal development stages when applied on egg-larval, pre-pupal or pupal stages as there was > 50% pre-imaginal development.

Other findings of the study are not presented here.

Conclusions: *Bacillus thuringiensis* var. *kurstaki* showed no significant persistent toxicity to the two trichogrammatid species tested.

Comments and conclusion RMS:

It was concluded that under the conditions of this study the *Bacillus thuringiensis* var. *kurstaki* was not toxic to T. sp. nr. *lutea* and T. sp nr. *mwanzai*. The results of the study can be used as supportive information.

Reference:

KIIM 8.4/09: Amichot, M., Curty, C., Bentuettat-Magliano, O., Gallet, A., Wajnberg, E. (2016)
Side effects of *Bacillus thuringiensis* var. *kurstaki* on the hymenopterous parasitic wasp *Trichogramma chilonis*
Environmental Science Pollution Research, 23, 3097-3103

Guideline: Not specified

GLP: No

Abstract Most of the detrimental effects of using conventional insecticides to control crop pests are now well identified and are nowadays major arguments for replacing such compounds by the use of biological control agents. In this respect, the bacterium *Bacillus thuringiensis* var. *kurstaki* and *Trichogramma* (Hymenoptera: Trichogrammatidae) parasitic wasp species are both effective against lepidopterous pests and can actually be used concomitantly. In this work, we studied the potential side effects of *B. thuringiensis* var. *kurstaki* on *Trichogramma chilonis* females. We first evidenced an acute toxicity of *B. thuringiensis* on *T. chilonis*. Then, after ingestion of *B. thuringiensis* sublethal doses, we focused on life history traits of *T. chilonis* such as longevity, reproductive success and the time spent on host eggs patches. The reproductive success of *T. chilonis* was not modified by *B. thuringiensis* while a significant effect was observed on longevity and the time spent on host eggs patches the physiological and ecological meanings of the results obtained are discussed.

Materials and Methods: The study was conducted in the laboratory. For evaluation of acute toxicity, *B. thuringiensis* var. *kurstaki* (Dipel ®) different amount of spores were incorporated into the insect food. For evaluation of longevity, isolated insects were fed with Btk preparation and checked daily for mortality. For evaluation of fecundity and parasitism success, *Ephestia kueniella* eggs were offered as host eggs and the number of emerging adults was counted after 4 - 5 days.

Other experiments of the study are not presented here.

Findings: In the assessment of acute toxicity, mortality was observed when females were fed with Dipel® (5×10^4 CFU/µg); the LC_{50} was calculated as 84.2 µg/µL (confidence limits: 9.5 - 288.5 µg/µL). The further tests for longevity and reproduction were performed with a lower dose of Btk of 4 µg/µL, representing about five times less than the LC_{10} value. The longevity of females fed with Btk spores was significantly longer than the one obtained with the control. Regarding the assessment of fecundity and parasitism, no differences in the number of adult *T. chilonis* emerging were recorded between the Btk preparation and the control.

Other findings of the study are not presented here.

Conclusions: No effect on host feeding behaviour, fecundity and parasitism success was observed. However, an effect on mortality and extended longevity was observed.

Comments and conclusion RMS:

The current study demonstrates that spores devoid of Cry toxins have no acute toxicity at all tested doses. Dipel preparations which include the Cry toxins had an acute toxicity against wasps. The authors do not exclude that some of the effects might be due to the co-formulants. The authors conclude that Btk can have different effects depending on the phylogeny of the insects tested and that the efficiency of *Trichogramma* as a biocontrol agent could be affected by the concomitant treatment with Dipel. The results of the current study can be used as a supplementary information.

Table B.9.3.5.b: Toxic effects of MPCA to arthropods other than bees

Test species	Toxicity
Uga menoni	30 day dietary LC_{50}: 4.4×10^7 CFU/g feed 30 day dietary NOEC: 10^7 CFU/g feed

Toxin/metabolite from microbial pest control agent (MPCA)

TOXIN/METABOLITE FROM ACTIVE INGREDIENT

The Bta strain GC-91 does not produce metabolites toxic for human health or the environment. This is confirmed by the quality control tests during the fermentation and production process, respectively. The working vials will be tested for the amount of ß-exotoxins in the fermentation broth by HPLC anal-

ysis, fly test and mice injection. Furthermore, after the fermentation process the spores will be spray dried while the nutrient broth is discarded. If any metabolites would occur, they would be removed from the Technical Powder consequently (Chen & Hargrove, 2003).

B.9.3.6 Infectiveness to arthropods other than bees

No signs of infectivity in *Euseius victoriensis* (predatory mite). Infectivity was not investigated in the tests (n.b. tests conducted with the product Agree 50 WG) with *Aphidius rhopalosiphi* and *Typhlodromus pyri .*

B.9.3.7 Pathogenicity to arthropods other than bees

No signs of pathogenicity in *Hyppodamia convergens* (ladybird beetle), *Euseius victoriensis* (predatory mite). Pathogenicity was not investigated in the tests (n.b. tests conducted with the product Agree 50 WG) with *Aphidius rhopalosiphi* and *Typhlodromus pyri.*

B.9.4 Effects on earthworms (Annex IIM 8.5; Annex IIIB 10.5)

B.9.4.1 Toxicity to earthworms

New data 2016

No substantial new information is submitted for renewal of the strain according to Regulation (EC) No 1107/2009. Instead it is referred to the study assessing the effects of formulation Agree WP on earthworms submitted for original strain approval. The literature search covering the last 10 years and focussing to target possible toxicity or pathogenicity of Bta on earthworms did not provide any relevant information.

The presented literature references were obtained in a general search for Bta and not by the search according to EFSA guidance and focussing on the risk assessment.

Due to available strain specific data and available knowledge about *Bacillus thuringiensis* subsp. *aizawai* in general it can be concluded that Bta GC-91 is not toxic, pathogenic or infective to earthworms.

Cited literature abstracts:

Report KMA 8.5/01 - Bilej, M., Procháuková, P., Šilerová, M., Josková, R. (2010) Earthworm Immunity

published report

Invertebrate Immunity, edited by Kenneth Söderhäll, Landes Bioscience and Springer Science+Business Media, p. 66 -79

Abstract: Earthworms belonging to oligochaete annelids became a model for comparative immunologists in the early sixties with the publication of results from transplantation experiments that proved the existence of self/nonself recognition in earthworms. This initiates extensive studies on the earthworm immune mechanisms that evolved to prevent invasion of pathogens. In the last four decades important cellular and humoral pathways were described and numerous biologically active compounds were characterized and often cloned.

Comments and conclusion RMS:

RMS agrees with the opinion of the applicant which is also in line with OECD 67.

B.9.4.2 Infectiveness to earthworms

Based on the information provided above, *Bta* is not expected to be infective to earthworms.

B.9.4.3 Pathogenicity to earthworms

Based on the information provided above, *Bta* is not expected to be pathogenic to earthworms.

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

According to the Working Document to the Environmental Safety Evaluation of Microbial Biocontrol Agents (SANCO/12117/2012)[4] tests assessing possible effects of microbial pesticides on soil micro-organisms are not stringently significant for the following reasons:

- Micro-organisms may be affected by almost everything that is added to the soil. Interpretation of test results is often ambiguous.

- Risk caused by introduction of micro-organisms to the soil microbial community is minimal, because soil microflora naturally fluctuates in time and space. The natural populations are well adapted to their habitat and exhibit many defence mechanisms in order to assure their survival.

[4] Working Document to the Environmental Safety Evaluation of Microbial Biocontrol Agents, SANCO/12117/2012-rev.0, September 2012, EUROPEAN COMMISSION HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL Directorate E – Safety of the food chain Unit E.3 – Chemicals, contaminants, pesticides.

- Soil microbial communities show good resilience, and populations are able to recover even upon extreme decimation e.g. by methyl bromide.

- Based on the available knowledge and on the experience that nitrification and respiration test performed with several microbial pest control agents, such as *Beauveria bassiana*, *Trichoderma* spp., or *Bacillus thuringiensis*, never showed adverse effects, it was concluded that the relevance of these tests is low.

Scheepmaker & van de Kassteele (2011) investigated the influence of microbial control agents on soil microbial communities. This study was based on data (CFU counts) available from the open literature, which were chosen following strict criteria regarding their usefulness and reliability. The quantitative effect of bacterial and fungal antagonists and chemical control agents on the total (culturable) number of different soil non-target organisms, soil bacteria, soil fungi, actinomycetes and protozoa was compared. The authors showed that microbial antagonists could have a short-term effect on the abundance of the fungal communities in soils. However, this effect was observed directly after the treatment only and complete recovery was demonstrated after 70 days at the latest. The initial effect on soil fungi was even more pronounced in the case of fungal antagonists than upon use of bacterial antagonists. Soil bacterial and protozoan communities were, in contrast to chemical treatments, not affected.

Due to available strain specific data and available knowledge about *Bacillus thuringiensis* subsp. *aizawai* in general it can be concluded that Bta strain GC-91 is not toxic, pathogenic or infective to soil micro-organisms.

Report: KMA 8.6/01 – O'Callaghan, M., Gerard, E., Sarathchandra, U. (2007)

Analysis of non-target impacts of Foray 48B on soil micro-organisms

Proceedings of the 6th Pacific Rim Conference on the Biotechnology of *Bacillus thuringiensis* and its Environmental Impact, 133-134

Guideline: Not specified

GLP: No

Abstract The effect of Foray 48B (*Bacillus thuringiensis* subsp. *kurstaki*, Btk) on indigenous soil micro-organisms was assessed in a pot trial in which four rates of Foray were applied. Foray had no impact on the genetic diversity of the indigenous soil eubacterial community, as measured by PCR-DGGE. Using *Bacillus*-specific PCR primers, bands corresponding to Btk were detected within the natural soil populations of bacilli only at 100 and 1000 × field rate (where field rate = 5 L/ha of Foray 48B). After 2 weeks, bacterial functional diversity (estimated by BIOLOG™ ecoplates) was similar in all treatments and total fungal and bacterial populations were greater in the 1000 × FR treatment only.

Materials and Methods: In a greenhouse trial, pots containing perennial ryegrass (*Lolium*

perenne) and white clover (*Trifolium repens*) grown in field collected soil were treated with Foray 48B (Abbott Laboratories) at four rates (0 – water only, 1 ×, 100 ×, and 1000 × field rate), where field rate was 5 L/ha (equivalent to 83.5 BIU ha^{-1}) and the effects on non-target soil micro-organisms were monitored using a polyphasic approach. Four replicate pots of each treatment were sampled at 1, 2 and 4 weeks after treatment application. Bacterial community DNA extracted from the soil and bacterial 16S rDNA fragments were amplified by PCR. PCR products were separated by denaturing gradient gel electrophoresis (DGGE).

Findings: DNA fingerprinting patterns showed that Foray 48B application had no impact on the diversity of the indigenous soil bacterial community. The soil bacterial functional diversity in pots treated with 1000 × FR was significantly different from the other treatments at 1 week after treatments, but after 2 weeks functional diversity was similar in all treatments (results not shown). Total culturable bacterial numbers did not differ significantly among treatments, with the exception of 1000 × FR, where bacterial numbers were significantly higher than in control soils.

Conclusions: Application of very high amounts of Foray 48B (1000 × FR) caused only transient effects on bacterial functional diversity and the total numbers of culturable bacteria and fungi. The addition of Foray 48B even at very high rates (1000 × FR) had no effect on diversity of predominant eubacterial populations present in soil, as determined by PCR-DGGE.

Cited literature abstracts:

Report KMA 8.6/02 - Scheepmaker, J. W. A., Van De Kassteele, J. (2011) Effects of chemical control agents and microbial biocontrol agents on numbers of non-target microbial soil organisms: a meta-analysis

published report

Biocontrol Science and Technology, 21, 1225-1242

Abstract: Our aim was to investigate the non-target effects on soil micro-organisms in agricultural environments caused by chemical control agents (CCAs) and microbial biocontrol agents (mBCAs), including the recovery from these effects. This was a desk study in which quantifiable endpoints, such as numbers of colony forming units (CFU), were derived from a series of studies and the combined data then analysed with a meta-regression analysis. Three analyses of the same dataset were performed. The first analysis, which was performed at the level of the CCAs and mBCAs in general, revealed that the effects of CCAs differed significantly from those of mBCAs. The second analysis, which included the type of non-target group (bacteria, fungi, actinomycetes, and protozoa) as additional input, revealed that mBCAs have greater effects than CCAs on fungi at study initiation, that CCAs had greater effects than mBCAs on bacteria and protozoa and that when effects were measured, recovery occurred within 100 days post-treatment initiation. The final analysis, which included the type of CCA (fungicide, insecticide, herbicide) or mBCA (antagonist) as additional input, revealed that (1) antagonists had a greater effect on fungi than insecticides and fungicides, (2) insecticides and to a lesser extent fungicides had a larger effect on bacteria than fungicides and antagonists, and (3) recovery of the CFU occurred within 100 days for all types of pesticides, mBCAs as well as CCAs, and for all non-target groups. The findings are discussed in view of the regulatory context of admittance of mBCAs to the market.

Comments and conclusion RMS:

RMS agrees with the opinion of the applicant which is also in line with OECD 67.

B.9.6 Effects on terrestrial plants

No information required as not a data requirement for micro-organisms.

B.9.7 Additional studies

No further studies are provided.

B.9.8 References relied on

Report Title: Literature review on *Bacillus thuringiensis* subsp. *aizawai* strain GC-91: Effects on non-target organisms

Guidelines: European Food Safety Authority; Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009 (OJ L 309, 24.11.2009, p. 1-50). EFSA Journal 2011;9(2):2092. [49 pp.]. doi:10.2903/j.efsa.2011.2092

GLP: no

This report summarizes the search and selection process of open peer-reviewed literature for *Bacillus thuringiensis* subsp. *aizawai* strain GC-91, in the framework of AIR4 active substance renewal process, with respect to possible effects on non-target organisms.

The strain Bta GC-91 is a transconjugant of a *Bacillus thuringiensis* subsp. *aizawai* (Bta) and a *Bacillus thuringiensis* subsp. *kurstaki* (Btk) strain. The literature search was extended to the subspecies *kurstaki*. It is also considered that Bta and Btk are closely related enough and are very similar with regard to their biological properties and physiological requirements. References on Btk are therefore considered fully applicable for the evaluation of Bta strain GC-91.

The search strategy was based on a multi-concept approach. For details regarding the search strategy and the results obtained, please refer to Point 4 and Point 5 of this Literature Review Report.

The selection process resulted in the classification of the available reports in the four categories recommended by the EFSA (2011)[5] guideline:

1) Studies that are relevant to the data requirement and that provide data for establishing or refining risk assessment parameters. These studies should be considered for reliability.
2) Studies that are relevant to the data requirement, but in the opinion of the applicant provide only supplementary information that does not alter existing risk assessment parameters.
3) Studies for which relevance cannot be clearly determined.
4) Studies of no relevance.

The relevance criteria applied are reported in Point 3.2 of this Literature Review Report.
The reliability assessment for relevant studies was done according to the recommendations of the EFSA (2011)[1].

The overall results are shown below on **Table 2-1**.

[5] Guidance of EFSA: 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)

Table 2-1 Results of the study selection process for Section 8

Data requirement capture in the search:	n
Total number of summary records retrieved after all searches of peer-reviewed literature	652
Number of summary records excluded from the search after rapid assessment for relevance	638
Total number of full-text documents assessed in detail	14
Number of studies excluded from further consideration after detailed assessment of relevance	4
Number of studies not excluded for relevance after detailed assessment	10

3 Protocol: objective of the review and criteria used

3.1 Objective

The review was made in order to identify scientific peer-reviewed open literature on the active substance *Bacillus thuringiensis* subsp. *aizawai* strain GC-91 which may affect the assessment on non-target organisms.

3.2 Criteria for relevance and reliability

The criteria for relevance and reliability used are summarised below in **Table 3.2-1.** Only studies that were considered relevant were assessed for reliability.

Table 3.2-1 Criteria for relevance and reliability used in the review

Relevance criteria
• Property investigated was relevant for data requirements of Regulation (EC) 1107/2009 • Subject relevant for ecotoxicological considerations? • Test species/system relevant to the ecotoxicological assessment? • Non-target organism not known as beneficial organism or described as pest? • Non-target organism relevant in the geographical location of intended use? • Route of administration / exposure relevant for assessment? • Endpoint relevant for the assessment? • Is the test substance relevant for the assessment? • Is the effect relevant from the species and up to the population level? • In the case of reports on known *Bacillus thuringiensis* subsp. *aizawai* and *Bacillus thuringiensis* subsp. *kurstaki* pathogens in a certain non-target organism, is there any relevance for *Bacillus thuringiensis* subsp. *aizawai* and *Bacillus thuringiensis* subsp. *kurstaki*?
Reliability criteria
Minimum information reported e.g.: • Test item or related compound • Test species relevant • Clear and comprehensive description of material and methods, incl. duration, replicates, test conditions • Definition of endpoints • Presentation of result • Guideline compliance

4 Search methods and results

The literature research was conducted on the DIMDI database provided by the German Institute of Medical Documentation and comprised searches in MEDLINE; BIOSIS, CAB Abstracts and SCISEARCH databases. Search strategy aimed to find all recent (from 2006 onwards) references that are of ecotoxicological relevance, regarding possible effects on non-target organisms.

Keywords used:

Active substance *Bacillus thuringiensis* subsp. *aizawai and kurstaki*, bird? aves?, fish? daphnid?, daphnia, alga?, aquatic plant?, phytotox?, phytopathogen?, bee, bees, honeybee, honeybees, bumblebees, arthropod?, insect?, tox?, pathogen?, transgen?, earthworm?, soil microorganism?

The “?” is used for the expansion of keywords.

Obtained references were first subjected to a *rapid assessment* based on title and the abstract. Summary records that appeared to be relevant passed to a second step in which a detailed assessment of full text documents was conducted.

Details on the search strategies are presented in **Table 4-1**. Results are listed in **Table 4-2**.

Table 4-1 Search process for peer-reviewed open literature in bibliographic databases

Database	BA00	CV72	SCISEARCH	MEDLINE
Justification for choosing the source	BIOSIS Previews covers worldwide literature in the field of biology (zoology, botany, microbiology), human and veterinary medicine, biochemistry, pharmacology, toxicology, and environmental sciences, especially from Northern America and Europe. It corresponds to the printed Biological Abstracts and Biological Abstracts/RRM (Reports, Reviews, Meetings). BA00: 2000 to date	CAB Abstracts covers worldwide literature of agriculture and related sciences including biotechnology, veterinary medicine, nutrition, medicine and forestry sciences. Sources include approx. 9,000 international journals, books, conference proceedings, and patents. CV72: 1972 to present	SciSearch covers worldwide literature in the fields of science, technology, and medicine. The database contains all citations published in "Science Citation Index Expanded". Sources include approx. 6,650 international journals of 150 disciplines including Clinical Medicine and Life Sciences. IS00: 2000 to date	MEDLINE (Medical Literature Analysis and Retrieval System Online) covers worldwide literature on every area of medicine, including dental medicine, veterinary medicine, psychology, and public health. The database corresponds to the printed "Index Medicus" and to some other printed material. Sources include approx. 4,800 international journals. ME00: 2000 to date
Date of the search	17.06.2016			
Date span of the search	2006-2016			
Language limit	English – Spanish – French – German			
Other limits set	Available abstracts			
Search type	Expert Search			
Search strategy Bta	Search term 1:	(*Bacillus thuringiensis* AND *aizawai* AND (bird? OR Aves))		
	Search term 2:	(*Bacillus thuringiensis* AND *aizawai* AND (fish? OR daphnid? OR daphnia OR (aquatic invertebrate?) OR alga? OR (aquatic plant?))		
	Search term 3:	(*Bacillus thuringiensis* AND *kurstaki* AND (phytotox? OR phytopathogen?)		
	Search term 4:	(*Bacillus thuringiensis* AND *aizawai* AND (bee OR bees OR honeybee OR honeybees OR bumblebee OR bumblebees))		
	Search term 5:	(*Bacillus thuringiensis* AND *aizawai* AND (arthropod? OR insect?) AND (tox? OR pathogen?) NOT (bee OR bees OR) NOT Bt-maize)		
	Search term 6:	(*Bacillus thuringiensis* AND *aizawai* AND earthworm?)		
	Search term 7:	(*Bacillus thuringiensis* AND *aizawai* AND (soil microorganism?))		
Search strategy Btk	Search term 1:	(*Bacillus thuringiensis* AND *kurstaki* AND (bird? OR Aves))		
	Search term 2:	(*Bacillus thuringiensis* AND *kurstaki* AND (fish? OR daphnid? OR		

		daphnia OR (aquatic invertebrate?) OR alga? OR (aquatic plant?))
	Search term 3:	(*Bacillus thuringiensis* AND *kurstaki* AND (phytotox? OR phytopathogen?)
	Search term 4:	(*Bacillus thuringiensis* AND *kurstaki* AND (bee OR bees OR honeybee OR honeybees OR bumblebee OR bumblebees))
	Search term 5:	(*Bacillus thuringiensis* AND *kurstaki* AND (arthropod? OR insect?) AND (tox? OR pathogen?) NOT (bee OR bees OR transgen?)
	Search term 6:	(*Bacillus thuringiensis* AND *kurstaki* AND earthworm?)
	Search term 7:	(*Bacillus thuringiensis* AND *kurstaki* AND (soil microorganism?))

* Use of „?“ at the end of keyword will lead to an expansion of the search criteria at DIMDI database

Table 4-2 Search results

Search strategy Bta	Total number of records	Total number after removal of duplicates
Search term 1	0	0
Search term 2	0	0
Search term 3	6	2
Search term 4	11	4
Search term 5	157	93
Search term 6	0	0
Search term 7	0	0
Total number of hits:		**99***

* Duplicate references identified by the title in several databases were deleted from the list.

Search strategy Btk	Total number of records	Total number after removal of duplicates
Search term 1	9	7
Search term 2	13	8
Search term 3	21	14
Search term 4	21	9
Search term 5	895	496
Search term 6	12	10
Search term 7	9	9
Total number of hits:		**553***

* Duplicate references identified by the title in several databases were deleted from the list.

5 Results of the study selection process

The relevance criteria were applied in the order presented in **Table 3.2-1** to sort out references that could be relevant (*rapid assessment* based on titles and abstracts).

Results were selected by evaluating and sorting all entries.

After the *rapid assessment*, 14 references were identified as being potentially relevant to subjected to a *detailed assessment* of the full-text documents.

The overall results of the literature research are presented in **Table 5-1.**

Table 5-1 Results of the study selection process

Data requirement capture in the search:	**n**
Total number of summary records retrieved after all searches of peer-reviewed literature	652
Number of summary records excluded from the search after rapid assessment for relevance	638
Total number of full-text documents assessed in detail	14
Number of studies excluded from further consideration after detailed assessment of relevance	4
Number of studies not excluded for relevance after detailed assessment	10

Table 5-2 and Table 5-3 below present all relevant studies subjected to a detailed assessment of full-text documents (n = 10) by data requirement and by author, respectively.

Table 5-4 presents all the studies excluded from the risk assessment after detailed assessment of full-text documents.

Table 5-2 List of bibliographical references identified as potentially relevant and studies of unclear relevance included in the dossier after detailed assessment of full-text documents for relevance: ordered by data requirements

EU point	Author	Year	Title	Source	Relevant to the data requirement?	Does the new information alter the risk assessments or List of Endpoints?	Reliability
MA 8.2.2	Oliveira-Filho, E.C., Muniz, D.H., Freire, I.S., Ramos F.R., Alves, R.T., Jonsson, C.M., Grisolia, C.K., Monnerat, R.G.	2011	Susceptibility on non-target invertebrates to Brazilian microbial pest control agents	Ecotoxicology, 20: 1354-1360	Yes	No	Yes
MA 8.3	del Mar-Leza, M., Llado, G., Petro, A.B., Alemany, A.	2014	First field assessment of *Bacillus thuringiensis* subsp. *kurstaki* aerial application on the colony performance of *Apis mellifera* (Hymenoptera: Apidae)	Spanish Journal of Agricultural Research, 12(2): 405-408	Yes	No	Yes
MA 8.3	Mommaerts, V., Jans, K., Smagghe, G.	2010	Impact of *Bacillus thuringiensis* strains on survival, reproduction and foraging behaviour in bumblebees (*Bombus terrestris*)	Pest Management Science, 66: 520-525	Yes	No	Yes
MA 8.4	Amichot, M., Curt, C., Benguettat-Magliano, O., Gallet, A., Wajnberg, E.	2016	Side effects of *Bacillus thuringiensis* var. *kurstaki* on the hymenopterous parasitic wasp *Trichogramma chilonis*	Environmental Science and Pollution Research, 23: 3097-3103	Yes	No	Yes
MA 8.4	Bernard, M.B., Cole, P., Kobelt, A., Horne, P.A., Altmann, J.,	2010	Reducing the impact of pesticides on biological control in Australian	BioOne, 103(6): 2061-2071	Yes	No	Yes

EU point	Author	Year	Title	Source	Relevant to the data requirement?	Does the new information alter the risk assessments or List of Endpoints?	Reliability
	Wratten, S.D., Yen, A.L.		vineyards: Pesticide mortality and fecundity effects on an indicator species, the predatory mite *Euseius victoriensis* (Acari: Phytoseiidae)				
MA 8.4	Carvalho, G.A., Moura, A.P., Bueno, V.H.P.	2006	Side effects of pesticides on *Trichogramma pretiosum* (Hymenoptera: Trichogrammatide)	IOBC/wprs Bulletin, 29(4): 355-359	Yes	No	Yes
MA 8.4	Garantonakis, N., Varikou, K., Birouraki, A.	2016	Comparative selectivity of pesticides used in greenhouses, on the aphid parasitoid *Aphidius colemani* (Hymenoptera: Braconidae)	Biocontrol Science and Technology, 26(5): 679-690	Yes	No	Yes
MA 8.4	Garcia, P.V., Pereira, N., Oliveira, L.M.	2009	Side-effects of organic and synthetic pesticides on cold-stored diapausing prepupae of Trichogramma cordubensis	BioControl, 54: 451-458	Yes	No	Yes
MA 8.4	Momanyi, G., Maranga, R., Sithanantham, S., Agong, S., Matoka, C.M., Hassan, S.A.	2012	Evaluation of persistence and relative toxicity of some pest control products to adults of two native trichogrammatid species in Kenya	BioControl, 57: 591-601	Yes	No	Yes
MA 8.6	O'Callaghan, M., Gerard, E., Sara-	2007	Analysis of non-target impacts of Foray 48B	Proceedings of the 6th Pacific Rim Confer-	Yes	No	Yes (with restriction –

EU point	Author	Year	Title	Source	Relevant to the data requirement?	Does the new information alter the risk assessments or List of Endpoints?	Reliability
	thchandra, U.		on soil micro-organisms	ence on the Biotechnology of *Bacillus thuringiensis* and its Environmental Impact 2007: 133-134			conference abstract only)

Table 5-3 List of bibliographical references identified as potentially relevant and studies of unclear relevance included in the dossier after detailed assessment of full-text documents for relevance: ordered by authors

Author	EU point	Year	Title	Source	Relevant to the data requirement?	Does the new information alter the risk assessments or List of Endpoints?	Reliability
Amichot, M., Curt, C., Benguettat-Magliano, O., Gallet, A., Wajnberg, E.	MA 8.4	2016	Side effects of *Bacillus thuringiensis* var. *kurstaki* on the hymenopterous parasitic wasp *Trichogramma chilonis*	Environmental Science and Pollution Research, 23: 3097-3103	Yes	No	Yes
Bernard, M.B., Cole, P., Kobelt, A., Horne, P.A., Altmann, J., Wratten, S.D., Yen, A.L.	MA 8.4	2010	Reducing the impact of pesticides on biological control in Australian vineyards: Pesticide mortality and fecundity effects on an indicator species, the predatory mite *Euseius victoriensis* (Acari: Phytoseiidae)	BioOne, 103(6): 2061-2071	Yes	No	Yes
Carvalho, G.A., Moura, A.P., Bueno, V.H.P.	MA 8.4	2006	Side effects of pesticides on *Trichogramma pretiosum* (Hymenoptera: Trichogrammatide)	IOBC/wprs Bulletin, 29(4): 355-359	Yes	No	Yes
del Mar-Leza, M., Llado, G., Petro, A.B., Alemany, A.	MA 8.3	2014	First field assessment of *Bacillus thuringiensis* subsp. *kurstaki* aerial application on the colony performance of *Apis mellifera* (Hymenoptera: Apidae)	Spanish Journal of Agricultural Research, 12(2): 405-408	Yes	No	Yes
Garantonakis, N., Varikou, K., Birouraki, A.	MA 8.4	2016	Comparative selectivity of pesticides used in greenhouses, on the aphid parasitoid *Aphidius colemani* (Hymenoptera: Braconidae)	Biocontrol Science and Technology, 5-6: 679-690	Yes	No	Yes
Garcia, P.V., Pereira, N., Oliveira, L.M.	MA 8.4	2009	Side-effects of organic and synthetic pesticides on cold-stored diapausing prepupae of	BioControl, 54: 451-458	Yes	No	Yes

Author	EU point	Year	Title	Source	Relevant to the data requirement?	Does the new information alter the risk assessments or List of Endpoints?	Reliability
			Trichogramma cordubensis				
Momanyi, G., Maranga, R., Sithanantham, S., Agong, S., Matoka, C.M., Hassan, S.A.	MA 8.4	2012	Evaluation of persistence and relative toxicity of some pest control products to adults of two native trichogrammatid species in Kenya	BioControl, 57: 591-601	Yes	No	Yes
Mommaerts, V., Jans, K., Smagghe, G.	MA 8.3	2010	Impact of *Bacillus thuringiensis* strains on survival, reproduction and foraging behaviour in bumblebees (*Bombus terrestris*)	Pest Management Science, 66: 520-525	Yes	No	Yes
O'Callaghan, M., Gerard, E., Sarathchandra, U.	MA 8.6	2007	Analysis of non-target impacts of Foray 48B on soil micro-organisms	Proceedings of the 6th Pacific Rim Conference on the Biotechnology of *Bacillus thuringiensis* and its Environmental Impact, 133-134	Yes	No	Yes (with restriction – conference abstract only)
Oliveira-Filho, E.C., Muniz, D.H., Freire, I.S., Ramos F.R., Alves, R.T., Jonsson, C.M., Grisolia, C.K., Monnerat, R.G.	MA 8.2.2	2011	Susceptibility on non-target invertebrates to Brazilian microbial pest control agents	Ecotoxicology, 20: 1354-1360	Yes	No	Yes

Table 5-4 List of bibliographical references excluded from the dossier after detailed assessment of full-text documents for relevance: ordered by authors

Author	Year	Title	Source	Relevant to the data requirements?	Does the new information alter the risk assessments or List of Endpoints?	Reliability	Justification
Hendriksen, N.B., Carstensen, J.	2013	Long-term survival of *Bacillus thuringiensis* subsp. *kurstaki* in a field trial	Canadian Journal of Microbiology, 59(1): 34-38	No	No	Yes	Field study to evaluate the fate of *Bacillus thuringiensis* subsp. *kurstaki* eight years (and the following 5 years) after spraying of 6.5 x 10^7cfu/ml. The area was monitored by sampling soil cores and determination of the bacteria in the soil homogenates via phase contrast microscopy, specific PCR primers for the plasmid encoded crystal protein active against Lepidopterans and random-primed PCR. This study has not been included in the dossier as it provides no supplementary information and does not alter the risk assessment. Furthermore, the study focused on the fate of *Bacillus thuringiensis* subsp. *kurstaki* under field conditions not on risks to non-target organisms.
Nour El-Deen, M.E.M., Abdallah, A.A.M.	2013	Effect of different compounds against *Tetranychus urticae* Koch and its predatory mite *Pyhtoseiulus persimilis* AH under laboratory conditions	Journal of Applied Sciences Research, 9(6): 3965-3973	No	No	No	This study is classified as not relevant and not supportive since the investigations focused on the effectivity of chemical insecticides to target organisms. Furthermore, the study is reliable with restrictions, since it was not conducted according to an internationally accepted testing guideline, and not well documented.
Renzi, M.T., Amichot, M., Pauron, D., Tchamitchian,	2016	Chronic toxicity and physiological changes induced in the honey bee by	Ecotoxicology and Environmental Safety, 127: 205-213	No	No	Yes	This study is classified as not relevant and not supportive since the investigations focused on the chronic toxicity and physiological changes and thus, the majority of endpoints obtained in the

Author	Year	Title	Source	Relevant to the data requirements?	Does the new information alter the risk assessments or List of Endpoints?	Reliability	Justification
S., Brunet, J.L., Kretzschmar, A., Maini, S., Belzunces, L.P.		the exposure to fipronil and *Bacillus thuringiensis* spores alone or combined					study cannot be used for risk assessment purposes.
Tenczar, E.G., Krischik, V.A.	2006	Management of Cottonwood leaf beetle (Coleoptera: Chrysomelidae) with a novel transplant soak and biorational insecticides to conserve coccinellid beetles	Journal of Economic Entomology, 99(1): 102-108	No	No	Yes	This study is classified as not relevant and not supportive since the investigations focused on the effectivity of insecticides (including *Bacillus thuringiensis* subsp. *kurstaki*) to target organisms.

Data point	Author(s)	Year	Title Owner, Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
ANNEX II DATA							
KMA 8.1/01	[redacted]	1990a	CGA-237218 TECHNICAL (GC-91) - AN AVIAN ORAL PATHOGENICITY AND TOXICITY STUDY IN THE BOBWHITE Certis USA LLC, 108-308 [redacted] GLP: yes Published: no	yes	no	not protected	CEU
KMA 8.1/02	[redacted]	1990b	CGA-237218 TECHNICAL (GC-91) - AN AVIAN ORAL PATHOGENICITY AND TOXICITY STUDY IN THE MALLARD Certis USA LLC, 108-309 [redacted] GLP: yes Published: no	yes	no	not protected	CEU
KMA 8.2.1/01	[redacted]	1991a	(CGA-237218 TECHNICAL MATERIAL) - INFECTIVITY AN PATHOGENICITY TO RAINBOW TROUT (ONCORHYNCHUS MYKISS) DURING A 32-DAY STATIC RENEWAL TEST Certis USA LLC, 90-6-3363 [redacted] GLP: yes Published: no	yes	no	not protected	CEU

Data point	Author(s)	Year	Title Owner, Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
ANNEX II DATA							
KMA 8.2.1/02	[redacted]	1991b	CGA-237218 - INFECTIVITY AND PATHOGENICITY TO SHEEPSHEAD MINNOW (CYPRINODON VARIEGATUS) DURING A 30-DAY STATIC RENEWAL TEST Certis USA LLC, 90-8-3439 [redacted] GLP: yes Published: no	yes	no	not protected	CEU
KMA 8.2.2/01	Collins, M.K.	1997	CGA-237218 ACUTE TOXICITY TO DAPHNIDS, DAPHNIA MAGNA, UNDER STATIC-RENEWAL CONDITIONS Certis USA LLC, 97-1-6842 Springborn Laboratories Inc., Massachusetts, USA GLP: yes Published: no	no	yes	protected	CEU
KMA 8.2.2/02	Christensen, K.P.	1991c	CGA-237218 CHRONIC TOXICITY TO DAPHNIDS (DAPHNIA MAGNA) UNDER STATIC RENEWAL CONDITIONS Certis USA LLC, 90-7-3385 Springborn Laboratories Inc., Massachusetts, USA GLP: yes Published: no	no	no	not protected	CEU

Data point	Author(s)	Year	Title Owner, Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
ANNEX II DATA							
KMA 8.2.2/03	Collins, M.K.	1993	INFECTIVITY AND PATHOGENICITY TO DAPHNIDS (DAPHNIA MAGNA) DURING A 21-DAY STATIC RENEWAL TEST Certis USA LLC, 93-10-4968 Springborn Laboratories Inc., Massachusetts, USA GLP: yes Published: no	no	no	not protected	CEU
KMA 8.2.2/04	Christensen, K.P.	1991d	CGA-237218 TECHNICAL MATERIAL - INFECTIVITY AND PATHOGENICITY TO GRASS SHRIMP (PALAEMONETES VULGARIS) DURING A 30-DAY STATIC RENEWAL TEST Certis USA LLC, 90-6-3445 Springborn Laboratories Inc., Massachusetts, USA GLP: yes Published: no	no	no	not protected	CEU
KMA 8.2.2/05	Oliveira-Filho, E.C., Freitas Muniz, D.H., Souza Freire, I., Ramos, F.R., Teixeira Alves, R., Jonsson, C.M., Koppe Grisolia, C., Gomes Monnerat, R.	2011	SUSCEPTIBILITY OF NON-TARGET INVERTEBRATES TO BRAZILIAN MICROBIAL PEST CONTROL AGENTS not available, not applicable Ecotoxicology, 20, 1354-1360 GLP/GEP: no Published: yes	no	no	not protected	-

Data point	Author(s)	Year	Title Owner, Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
ANNEX II DATA							
KMA 8.2.3/01	Grade, R.	1993	REPORT ON THE GROWTH INHIBITATION TEST OF CGA 237218 TECH. TO GREEN ALGAE (SCENEDESMUS SUBSPICATUS) Certis USA LLC, 938007 CIBA-GEIGY Ltd., CH-4002 Basel, Switzerland GLP: yes Published: no	no	no	not protected	CEU
KMA 8.3/01	Winter, P.A., Hoxter, K.A., Smith, G.J.	1991a	CGA-237218 A DIETARY PATHOGENICITY AND TOXICITY STUDY WITH THE HONEY BEE Certis USA LLC, 108-310A Wildlife International, Ltd., Easton, Maryland, USA GLP: yes Published: no	no	no	not protected	CEU
KMA 8.3/02	Parrish, J.R, Yeager, B.	1994	HONEY BEE TOXICITY FEEDING TEST/CHRONIC- CGA-237218 CERTIS USA LLC, HB419 BIO/WEST, INC., UTAH 84321, USA GLP: YES PUBLISHED: NO	no	no	not protected	CEU
KMA 8.3/03	Mommaerts, V., Jans, K., Smagghe, G.	2010	IMPACT OF BACILLUS THURINGIENSIS STRAINS ON SURVIVAL, REPRODUCTION AND FORAGING BEHAVIOUR IN BUMBLEBEES (BOMBUS TERRESTRIS) not available, not applicable Pest Management Science, 66, 520-525 GLP/GEP: no Published: yes	no	no	not protected	-

Data point	Author(s)	Year	Title Owner, Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
ANNEX II DATA							
KMA 8.3/04	del Mar Leza, M., Llado, G., Petro, A.B., Alemany, A.	2014	FIRST FIELD ASSESSMENT OF BACILLUS THURINGIENSIS SUBSP. KURSTAKI AERIAL APPLICATION ON THE COLONY PERFORMANCE OF APIS MELLIFERA L. (HYMENOPTERA:APIDAE) not available, not applicable Spanish J. of Agricult. Research, 12, 405-408 GLP/GEP: no Published: yes	no	no	not protected	-
KMA 8.4/01	Thompson, M.M., Hoxter, K.A., Smith, G.J.	1991	CGA-237218 A DIETARY PATHOGENICITY AND TOXICITY STUDY WITH THE GREEN LACEWING LARVAE Certis USA LLC, 108-312 Wildlife International, Ltd., Easton, Maryland, USA GLP: yes Published: no	no	no	not protected	CEU
KMA 8.4/02	Winter, P.A., Hoxter, K.A., Smith, G.J.	1991b	CGA-237218 A DIETARY PATHOGENICITY AND TOXICITY STUDY WITH THE PARASITIC HYMENOPTERAN UGA MENONI Certis USA LLC, 108-311A Wildlife International, Ltd., Easton, Maryland, USA GLP: yes Published: no	no	no	not protected	CEU

Data point	Author(s)	Year	Title Owner, Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
ANNEX II DATA							
KMA 8.4/03	Thompson, M.M., Hoxter, K.A., Jaber, M.	1991	CGA-237218 A DIETARY PATHOGENICITY AND TOXICITY STUDY WITH LADYBIRD BEETLES Certis USA LLC, 108-313 Wildlife International, Ltd., Easton, Maryland, USA GLP: yes Published: no	no	no	not protected	CEU
KMA 8.4/04	Bernard, M.B., Cole, P., Kobelt, A., Horne, P.A., Altmann, J., Wratten, S.D., Yen, A.L.	2010	REDUCING THE IMPACT OF PESTICIDES ON BIOLOGICAL CONTROL IN AUSTRALIAN VINEYARDS: PESTICIDE MORTALITY AND FECUNDITY EFFECTS ON AN INDICATOR SPECIES, THE PREDATORY MITE EUSEIUS VICTORIENSIS (ACARI: PHYTOSEIIDAE) not available, not applicable Entomol Society of America, 103(6), 2061-2071 GLP/GEP: no Published: yes	no	no	not protected	-
KMA 8.4/05	Carvalho, G.A., Moura, A.P., Bueno, V.H.P.	2006	SIDE EFFECTS OF PESTICIDES ON TRICHOGRAMMA PRETIOSUM (HYMENOPTERA: TRICHOGRAMMATIDAE) not available, not applicable IOBC/wprs Bulletin, 29 GLP/GEP: no Published: yes	no	no	not protected	-

Data point	Author(s)	Year	Title Owner, Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
ANNEX II DATA							
KMA8.4/06	Garcia, P.V., Pereita, N., Oliveira, L.M.	2008	SIDE-EFFECTS OF ORGANIC AND SYNTHETIC PESTICIDES ON COLD-STORED DIAPAUSING PREPUPAE OF TRICHOGRAMMA CORDUBENSIS not available, not applicable BioControl, 54, 451-458 GLP/GEP: no Published: yes	no	no	not protected	-
KMA 8.4/07	Garantonakis, N., Varikou, K., Birouraki, A.	2016	COMPARATIVE SELECTIVITY OF PESTICIDES USED IN GREENHOUSES, ON THE APHID PARASITOID APHIDIUS COLEMANI (HYMENOPTERA: BRACONIDAE) not available, not applicable Biocontrol Science and Technology, 26, 678-690 GLP/GEP: no Published: yes	no	no	not protected	-
KMA 8.4/08	Momanyi, G., Maranga, R., Sithanantham, S., Agong, S., Matoka, C.M., Hassan, S.A.	2012	EVALUATION OF PERSISTENCE AND RELATIVE TOXICITY OF SOME PEST CONTROL PRODUCTS TO ADULTS OF TWO NATIVE TRICHOGRAMMATID SPECIES IN KENYA not available, not applicable not available GLP/GEP: no Published: no	no	no	not protected	

Data point	Author(s)	Year	Title Owner, Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
ANNEX II DATA							
KMA 8.4/09	Amichot, M., Curty, C., Benguettat-Magliano, O., Gallet, A., Wajnberg, E.	2015	SIDE EFFECTS OF BACILLUS THURINGIENSIS VAR. KURSTAKI ON THE HYMENOPTEROUS PARASITIC WASP TRICHOGRAMMA CHILONIS not available, not applicable Environmental Science & Pollution Research 2 (2), 23, 3097-3103 GLP/GEP: no Published: yes	no	no	not protected	-
KMA 8.5/01	Bilej, M., Prochazkova, P., Silerova, M., Joskova, R.	2010	EARTHWORM IMMUNITY not available, not applicable Invertebrate Immunity, Kenneth Söderhäll (ed), 66-79 GLP/GEP: no Published: yes	no	no	not protected	-
KMA 8.6/01	O'Callaghan, M., Gerard, E., Sarathchandra, U.	2007	ANALYSIS OF NON-TARGET IMPACTS OF FORAY 48B ON SOIL MICRO-ORGANISMS not available, not applicable BioControl, 6, 133-134 GLP/GEP: no Published: yes	no	no	not protected	-

Data point	Author(s)	Year	Title Owner, Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
ANNEX II DATA							
KMA 8.6/02	Scheepmaker, J. W. A., van de Kassteele, J.	2011	EFFECTS OF CHEMICAL CONTROL AGENTS AND MICROBIAL BIOCONTROL AGENTS ON NUMBERS OF NON-TARGET MICROBIAL SOIL ORGANISMS: A META-ANALYSIS not available, not applicable Biocontrol Science and Technology, 21, 1225-1242 GLP/GEP: no Published: yes	no	no	not protected	-

Comment RMS: RMS agrees with the search terms and the data bases used by the applicant.