European Commission

Draft Renewal Assessment Report prepared according to the Commission Regulation (EU) N° 1107/2009

Microbial Pest Control Agent (MPCA) Bacillus thuringiensis subsp. *kurstaki* SA-12

Volume 3 B.9 (MPCA)

Effects on non-target organisms

Rapporteur Member State: Denmark

Co- Rapporteur Member State: The Netherlands

Version history

When	What
2008	DAR
2011	Addendum to the DAR
2019	Initial RAR

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B.9 Effects on non-target organisms

INTRODUCTION

Bacillus thuringiensis subsp. *kurstaki* SA-12 (in the following abbreviated as Btk SA-12) 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 Denmark as rapporteur Member State to carry out the assessment of Btk SA-12 on the basis of a joint dossier submitted for the Btk strains SA-11, SA-12 and EG 2348. The notifier for Btk SA-11 and SA-12 was Mitsui AgriScience International SA/NV while EG 2348 was notified by Mitsui AgriScience International SA/NV and Intrachem Bio Italia S.p.A. (now CBC (Europe) S.r.l.). In accordance with the provisions of Article 22(1) of Regulation (EC) No 2229/2004, Denmark submitted in January and February 2008 to the EFSA the draft assessment report, including, as required, a recommendation concerning the possible inclusion of Btk SA-12 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 12 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 finalized in the meeting of the Standing Committee on 12 July 2008. Subsequently Regulation (EC) No 1107/2009 repealed and replaced Directive 91/414/EEC and the active substance Btk SA-12, was deemed to be approved under that Regulation and included in the Annex to Regulation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. *kurstaki* (strains ABTS-351, PB-54, SA-11, SA-12, EG2348) on the 16 December 2011 (published 23 February 2012). Based on this new information available, no need to change the conditions of approval of Btk SA-12 was identified. The Commission filed on 13 December 2013 an updated review report for Btk strains SA-11, SA-12 and EG 2348 to the Standing Committee on the Food Chain and Animal Health for examination.

The approval of Btk SA-12 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 Btk SA-12 on 30 April 2016. In accordance with Regulation (EU) 2016/183 the notifier submitted to the designated RMS Denmark, the co-RMS The Netherlands as well as to EFSA and Commission a dossier for renewal of Btk SA-12 considering the deadline stated in SANTE-2016-10616–rev. 3.

Btk SA-12 is a wild type strain originating from infested insects. Btk 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 Btk is mainly attributed to spore bound insecticidal pro-proteins (*Cry* toxins) which are ingested by the target pests and activated under alkaline conditions in the midgut of the larvae. The first assessment of the strain proved that it does not have any harmful effects on human or animal health or on groundwater or any unacceptable influence on the environment. The overall conclusion from EFSA (2012) confirms that no critical areas of concern are identified within the framework of the use which was supported.

For the renewal of the Btk strains SA-11, SA-12 and EG 2348 under Regulation (EC) 1107/2009, a separate dossier was submitted for each strain only including data, which have previously not been submitted or evaluated. Nevertheless, there is some information which is applicable to all three Btk strains, e.g. published information for Btk in general obtained during searches for peer reviewed literature according to EFSA Guidance (2011)[1] carried out for relevant sections.

In the following for ease of information, full study summaries/sections taken from the DAR (2008) or its Final Addendum (2011) are included if they are considered relevant for renewal of Btk SA-12. In order to facilitate discrimination between new data and data already evaluated during the first approval process, the headline "New Data" begins the section with data, which have previously not been submitted or evaluated. Data and their evaluations from the original DAR and addenda to the DAR are highlighted by grey background.

The representative formulation for renewal of the approval of Btk SA-12 under Regulation (EC) 1107/2009 is CoStar WG. CoStar WG is a WG formulation having a biopotency of 90000 IU/mg. The content of the active ingredient is 85% corresponding to a maximum of 5.7×10^{13} CFU/kg product. CoStar WG was not the representative formulation for original approval of the strain. Therefore, no data have been submitted for this formulation before. However, CoStar WG, except for the active ingredient, is identical to the representative formulation for original approval, Delfin WG, containing Btk SA-11. Also the two Btk strains are very similar with regard to

1 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

their biological properties and physiological requirements. It is therefore justified to use data for Delfin WG also for the evaluation of CoStar WG. In addition, the manufacturing process of SA-12 has not been changed since original approval all data previously submitted and referring to Btk SA-12 are considered fully applicable for the current evaluation.

For renewal, representative uses chosen for renewal of Btk SA-12 cover control of *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. Application rates range between 1 – 2 kg with 6 subsequent applications at an interval of 7 days.

It is considered that the Critical GAP of CoStar WG chosen for the renewal of the active substance Btk SA-12 covers worst case exposure scenarios for human, non-target organisms and the environment.

Critical GAP of CoStar WG for renewal of Btk SA-12

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 67-89	a) 6 (7) b) 6 (7)	a) 1.5 b) 9.0	a) 1275 / 1.35 × 10^{11} b) 7650 / 8.1 × 10^{11}	1000-1500
Tomato	G	*Tuta absoluta*	Foliar spray	BBCH 12-89	a) 6 (7) b) 6 (7)	a) 1.0 b) 6.0	a) 850 / 9.0 × 10^{10} b) 5100 / 5.4 × 10^{11}	200-1000
Turf, Sports	F	*Spodoptera* spp.	Foliar spray	BBCH 12-89	a) 6 (7) b) 6 (7)	a) 2.0 b) 12.0	a) 1700 / 1.8 × 10^{11} b) 10200 / 1.1 × 10^{12}	500-1000

Biopotency of CoStar WG: 90000 IU/mg
Max. CFU content in CoStar WG: 5.7 × 10^{13} CFU/kg

A literature search according to EFSA (2011)[1] was conducted in May 2016 covering the last 10 years. 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 of *Bacillus thuringiensis* subsp. *kurstaki* on non-target organisms. After rapid assessment based on title and abstract; 14 references were submitted to full text analysis. In total, 10 references were considered relevant and reliable and are summarised under the respective data points below. For more details please refer to Schöbinger (2016, Document KMA 8.1/01).

B.9.1 Effects on birds

No strain specific data for Btk SA-12 was provided for first approval, but data for the closely related Btk strain SA-11 was considered acceptable to cover the requirements for first approval of the strain. Please refer to the DAR 2008.

New data

For renewal of Btk SA-12, a study is submitted assessing the side effects of Thuricide SC on birds. Although the study was conducted with a liquid formulation of Btk SA-12 it is considered relevant for the evaluation of the strain as it follows OPPTS guidance (now called OCSPP guidance) for testing of microbial active substances. More details on the formulation of the product are provided in the confidential Volume 4. According to the guidance testing of one herbivorous and one insectivorous bird species is recommended. As Btk SA-12 is used as an insecticide insectivorous birds are considered most relevant and represent the worst-case exposure situation. Btk-SA12 does not grow endophytic and no (relevant) residues on crop are expected. There is no indication for toxicity or pathogenicity in the study. For reasons of animal welfare it is not justified to perform a study on a second, herbivorous, species.

Report:	KMA 9.1/03 - [redacted](2015a)
Title:	Thuricide SC: Avian (*Coturnix coturnix japonica*) oral acute toxicity/pathogenicity study
Document No:	Report No. RL1364/2015PAVO-B
Guidelines:	OPPTS 885.4050 Avian oral, Tier I, 1996
GLP	Yes
Validity	Yes

Executive summary

Thuricide SC was administered to 13-day old female birds by oral gavage at a constant volume of 5 mL/kg bw corresponding to a dose level of 5.0×10^9 cfu/kg bw for a five day period. Two control groups (inactivated control at a dose level of 5.0×10^9 cfu/kg bw and a negative control) were also included in the test.

There were no clinical signs of toxicity or mortality observed during the observation period of 30 days. No evidence of pathogenicity or replication of the test substance was observed during gross necropsy at the termination of the test.

The test substance Thuricide SC was classified as non-toxic and non-pathogenic and the estimated oral acute LD_{50} in Japanese quail was considered to be higher than 5.0×10^9 cfu/kg bw.

MATERIAL AND METHODS

Test Item

Designation	Thuricide SC
Active ingredient	*Bacillus thuringiensis* subsp. *kurstaki* SA-12
Purity	5.0×10^9 cfu/mL (19%)
Characteristics	liquid
Batch no.	001-14-14400
Expiration date	August 2016

Test System

Species	Japanese quail (*Coturnix coturnix japonica*)
Age	13 days old females

Source	[redacted]
Number	10 birds per replicate
Acclimatisation period	5 days in a climate-controlled room prior to dosing

Test Conditions

Housing	Groups of 10 birds
Food	Game bird ration formulated by Wildlife International Ltd *ad libitum*. Vitamin supplemented water *ad libitum*.
Temperature	24 - 26°C
Photoperiod	16 hours of light per day
Humidity	51 - 59%

Study Design and Methods

In-life dates	22.06.2015 to 22.07.2015
Experimental treatment	Fifty young Japanese quail were distributed in 5 pens with ten birds per pen. All birds were individually weighed and administered the appropriate dosing suspension using a syringe. One replicate of 10 birds received oral doses of deionized water at 5 mL/kg bw/day for five days. One replicate of ten birds received oral doses of inactivated test substance at 5.0×10^9 cfu/kg bw/day for five days. Three replicates of ten birds received oral doses of Thuricide SC at 5.0×10^9 cfu/kg bw/day for five days.
Observations	Birds were observed daily during the test period, and then weekly until the end of the test (day 30) regarding mortality, signs of toxicity and abnormal behaviour.

RESULTS AND DISCUSSION

During the observation period of 30 days, no clinical sign of toxicity or mortality were observed in the treated groups and in the controls. No bird from the controls and treated groups showed any abnormal behaviour or appearance. All treated groups showed body weight gain at the end of the trial period. Necropsy of all other birds was unremarkable.

CONCLUSIONS

Under the test conditions, the test substance Thuricide SC was classified as non-toxic and non-pathogenic, and the estimated oral LD_{50} for Japanese quails was considered to be higher than 5.0×10^9 cfu/kg bw.

Table 9.1-1. Toxicity effects/ Infectivity / Pathogenicity of the Btk SA-12 to bird

Test species	Japanese quail (*Coturnix coturnix japonica*)
Toxicity	$LD_{50} > 5.0 \times 10^9$ CFU/kg b.w./
Infectivity / Pathogenicity	Not pathogenic Infectivity not determined in studies

Comments and conclusion RMS:

Although the study was conducted with a liquid formulation of Btk SA-12 it is considered relevant for the evaluation of the strain since the co-formulants are inert. The study follows guideline OPPTS 885.4050 (1996) (now called OCSPP guidance) for testing of microbial active substances. Under the test conditions, the test substance Thuricide SC was classified as non-toxic and non-pathogenic, and the estimated oral LD_{50} for Japanese quails was considered to be higher than 5.0×10^9 cfu/kg bw. 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.

Information from open literature

In addition, in Canada, Buckner *et al.* (1974) assessed the impact of the formulations DiPel and Thuricide, both containing Btk, on breeding bird populations (74 species representing 21 families) during a field trial for spruce budworm control. The bird populations in control and treated plots were measured before aerial application and for up to three weeks following application. No significant differences were detected between the populations in the control and treated plots, and thus, application of Btk formulations was considered not to have any effect on resident bird populations.

In a further assessment of the effects on birds in Arkansas in the USA, Nagy & Smith (1997) found that after a reduction in the population of lepidopteron larvae following two applications of a Btk formulation in one year, only very minimal effects on reproduction in Hooded warblers occurred in the breeding season the year following application.

The data confirms that birds will not be affected by insecticidal crystal proteins and living spores of *B. thuringiensis kurstaki.*

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

RMS evaluation	No strain specific data for Btk SA-12 was provided for first approval, but data for the closely related Btk strain SA-11 was considered acceptable to cover the requirements. For renewal of Btk SA-12, a study is submitted assessing the side effects of Thuricide SC on birds. Under the test conditions, the test substance Thuricide SC was classified as non-toxic and non-pathogenic, and the estimated oral LD_{50} for Japanese quails was considered to be higher than 5.0×10^9 cfu/kg bw. No signs of treatment related pathogenicity or toxicity were observed. According to the guidance testing of one herbivorous and one insectivorous bird species is recommended. As Btk SA-12 is used as an insecticide insectivorous birds are considered most relevant and represent the worst-case exposure situation. The study is considered relevant and reliable. The endpoint can be used in risk assessment. With respect to the GAP use of the Btk SA-12 formulation for renewal, the establishment of a reliance of birds on the target organisms (*Cydia pomonella* and *Spodoptera* spp.) as main source of diet is not expected to occur. The Btk formulation is expected to cause a quick and effective control and reduce any populations of the target organism occurring in fields, while maintaining other non-target insects in the field. The literature search covering the last 10 years and focussing to target possible toxicity or pathogenicity of Btk to birds did not provide any relevant information. Due to strain specific data presented above and available knowledge about *Bacillus thuringiensis* subsp. *kurstaki* in general it can be concluded that Btk SA-12 is not toxic, pathogenic or infective in birds. In conclusion no adverse effects are expected in birds upon field application of Btk SA-12 and further data are not required.
Endpoint: Effects on birds	$LD_{50} > 5.0 \times 10^9$ CFU/kg b.w./day

B.9.2 Effects on aquatic organisms

B.9.2.1 Effects on fish

No strain specific data for Btk SA-12 was provided for first approval, but data for the closely related Btk strain SA-11 was considered acceptable to cover the requirements for first approval of the strain. Please refer to the DAR 2008.

New data

For renewal of Btk SA-12, a study is submitted assessing the side effects of Thuricide SC on fish. Although the study was conducted with a liquid formulation of Btk SA-12, it is considered relevant for the evaluation of the strain as it follows OPPTS guidelines (since 2010 known as OCSPP guidelines) for testing of microbial active substances. More details on the formulation of the product are provided in the confidential part volume 4. The study follows OPPTS guidelines (since 2010 known as OCSPP guidelines) for testing of microbial active substances. *Danio rerio* is not the preferred species according to OCSPP 885.4200. In the guidance the cold-water species rainbow trout is mentioned. For microorganisms higher temperatures should potentially be considered more favourable for growth. Therefore, the study is considered to represent worst-case exposure conditions. There is no indication for toxicity or pathogenicity in the study. For reasons of animal welfare it is not justified to perform a study on a second, cold water, species. In addition, an acute toxicity study following OECD guidance with the formulated product is available. For the study summary, please refer to Vol. MP, Section B.9, Point B.9.2.1.

Report:	KMA 9.2.1/01 - [redacted] (2015b)
Title:	Thuricide SC: Fish (*Danio rerio*) toxicity test
Document No:	Report No. RL1371/2015PX-B
Guidelines:	OPPTS 885.4200 Freshwater fish testing, Tier I, 1996
GLP	Yes
Validity	Yes

Executive summary

The possible adverse effects on fish (*Danio rerio*) were evaluated over a test period of 30 days under semi-static conditions. The test was conducted with a nominal concentration of 5.0×10^6 cfu/mL; two control groups (inactivated control at a dose level of 5.0×10^6 cfu/mL and a negative control) were also included in the test.

After 30 days, no mortality was observed in the negative control and the test item group. 7% mortality was observed at the inactivated control group. No abnormal responses were observed after 30 days at all test groups.

After 30 days of exposure, the median lethal concentration of Thuricide SC (30-d LC_{50}) under the test conditions and based on nominal concentration was considered to be greater than 5.0×10^6 cfu/mL.

MATERIAL AND METHODS

Test Item

Designation	Thuricide SC
Active ingredient	*Bacillus thuringiensis* subsp. *kurstaki* SA-12
Purity	5.0×10^9 cfu/mL (19%)
Characteristics	liquid
Batch no.	001-14-14400
Expiration date	August 2016

Test System

Species	*Danio rerio* (Hamilton-Buchanan, 1822)
Source	[redacted]
Number	10 fish per replicate
Length	1.4 ± 0.2 cm
Weight	0.066 ± 0.049 g
Acclimatisation period	22 days at test conditions
Food	Tetramin Tropical Flakes

Test Conditions

Temperature	21.2 - 25.7°C
Photoperiod	16 hours of light per day and 8 hours darkness
Oxygen content	≥ 40% of the air saturation value
Hardness	41.0 - 43.0 mg $CaCO_3$/L
pH	7.02 - 7.87 at test start and 7.30 - 7.52 at test termination

Study Design and Methods

In-life dates	26.05.2015 to 25.06.2015
System	Semi static (one complete substitution per week)
Duration	30 days
Test vessel	3000 mL beakers
Concentration	5.0×10^6 cfu/mL, inactivate control at 5.0×10^6 cfu/mL, negative control
	3 replicates per test concentration and controls
Aeration	Until oxygen saturation was achieved
Observations	Fish were observed daily during the test period until the end of the test (day 30) regarding mortality, signs of toxicity and abnormal behaviour.

RESULTS AND DISCUSSION

After 30 days, no mortality was observed in the negative control and the test item group. 7% mortality was observed at the inactivated control group. No abnormal responses were observed after 30 days at all test groups.

Table 9.2.1-1 Mortality (%) of *Danio rerio* after 30 days of exposure to Thuricide SC

	Number of fish introduced	Number of dead fish	Mortality (%)
Negative control	30	0	0
Inactivated control	30	2	7
1.00×10^6 cfu/mL	30	0	0

CONCLUSIONS

The median lethal concentration of the test substance Thuricide SC after 30 days exposure (30-d LC_{50}) under the test conditions and based on a nominal concentration was considered greater than 5.0×10^6 cfu/mL.

Table 9.2.1-2 Toxicity effects/ Infectivity / Pathogenicity of the Btk SA-11 to fish

Test method	**Test species**	**Dose range Btk strain tested**	**Observations**	**Results/Endpoint**	**Reference**
30-day, semi-static	Fish (*Danio rerio*)	Thuricide SC (SA-12) 5.0×10^6 cfu/mL	After 30 days, no mortality was observed in the negative control and the test item group. 7% mortality was observed at the inactivated control group. No abnormal responses were observed after 30 days at all test groups. Infectivity was not investigated. No signs of pathogenicity or toxicity	$LC_{50} > 5.0 \times 10^9$ CFU/L	KMA 9.2.1/01 [redacted] (2015b)

* based on nominal concentrations

Comments and conclusion RMS:

Although the study was conducted with a liquid formulation of Btk SA-12 it is considered relevant for the evaluation of the strain since the co-formulants are inert. The study follows guideline OPPTS 885.4200 Freshwater fish testing, Tier I, 1996 for testing of microbial active substances. After 30 days, no mortality was observed in the negative control and the test item group. 7% mortality was observed at the inactivated control group. No abnormal responses were observed after 30 days at all test groups. After 30 days of exposure, the median lethal concentration of Thuricide SC (30-d LC_{50}) under the test conditions and based on nominal concentration was considered to be greater than 5.0×10^6 cfu/mL. 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.

Information from open literature

No information from open litterature was provided for first approval. The literature search covering the last 10 years and focussing to target possible toxicity or pathogenicity of Btk to fish did not provide any relevant information.

RMS evaluation	No strain specific data for Btk SA-12 was provided for first approval, but data for the closely related Btk strain SA-11 was considered acceptable to cover the requirements. For renewal of Btk SA-12, a study is submitted assessing the side effects of Thuricide SC on fish. Use of a warm-water species is justified for testing of a microorganism as higher temperatures are more favourable for microbial growth and thus represent worst-case exposure conditions. The median lethal concentration of the test substance Thuricide SC after 30 days exposure under the test conditions and based on a nominal concentration was considered greater than 5.0×10^6 cfu/mL. The endpoints can be used in risk assessment. The literature search covering the last 10 years and focussing to target possible toxicity or pathogenicity of Btk to fish did not provide any relevant information. Due to strain specific data presented above and available knowledge about *Bacillus thuringiensis* subsp. *kurstaki* in general it can be concluded that Btk SA-12 is not toxic, pathogenic or infective in fish (Table 9.2.1-1).
Endpoint: Effects on fish	$LC_{50} > 5.0 \times 10^9$ CFU/L

B.9.2.2 Effects on freshwater invertebrates

No strain specific data for Btk SA-12 was provided for first approval, but data for the closely related Btk strain SA-11 was considered acceptable to cover the requirements. Please refer to the DAR 2008.

New data 2016

For renewal of Btk SA-12, a study is submitted assessing the side effects of Thuricide SC on *Daphnia magna*. Although the study was conducted with a liquid formulation of Btk SA-12 it is considered relevant for the evaluation of the strain as it follows OPPTS guidance (now called OCSPP guidance) for testing of microbial active substances. More details on the formulation of the product are provided in the confidential part of the dossier (Volume 4). In addition, an acute toxicity study following OECD guidance with the formulated product CoStar WG is available. For the study summary please refer to Vol. 3 MP, Section B.9, Point B.9.2.2.

Report:	KMA 9.2.2/05 - Mrotzeck Masquetto, A. (2015)
Title:	Thuricide SC: *Daphnia magna* toxicity test
Document No:	Report No. RL1339/2015DP-B
Guidelines:	OPPTS 885.4240 Freshwater aquatic invertebrate testing, Tier I, 1996
GLP	Yes
Validity	Yes

Executive summary

The possible adverse effects on *Daphnia magna* were evaluated over a test period of 21 days under semi-static conditions. The test was conducted with a nominal concentration of 1.0×10^6 cfu/mL; two control groups (inactivated control at a dose level of 1.0×10^6 cfu/mL and a negative control) were also included in the test.

After 21 days, 4% mortality was observed in the negative control. 12% mortality was observed at the inactivated control group and the test item group at 1.0×10^6 cfu/mL. The mean number of juveniles produced per female at 1.0×10^6 cfu/mL was 80 juveniles/female in the negative control, 134 juveniles/female in the test item group and 107 juvenile/female in the inactivated control.

After 21 days of exposure, the median lethal concentration of Thuricide SC (21-d LC_{50}) under the test conditions and based on nominal concentration was considered to be greater than 1.0×10^6 cfu/mL.

MATERIAL AND METHODS

Test Item

Designation	Thuricide SC
Active ingredient	*Bacillus thuringiensis* subsp. *kurstaki* SA-12
Purity	5.0×10^9 cfu/mL (19%)
Characteristics	liquid
Batch no.	001-14-14400
Expiration date	August 2016

Test System

Species	*Daphnia magna* (Cladocera, Crustacea)
Source	Rearing culture at the testing facility
Number	10 per replicate
Food	Algal suspension and compound feed

Test Conditions

Temperature	18.6 ± 0.6°C
Photoperiod	16 hours of light per day and 8 hours darkness
Oxygen content	Not specified
Hardness	186.5 - 205.0 mg $CaCO_3$/L
pH	7.67 - 8.55 at test start and 6.85 - 7.57 at test termination

Study Design and Methods

In-life dates	26.05.2015 to 16.06.2015
System	Semi static (one complete substitution per week)
Duration	21 days
Test vessel	250 mL beakers
Concentration	1.0×10^6 cfu/mL, inactivate control at 1.0×10^6 cfu/mL, negative control

	5 replicates per test concentration and controls
Aeration	None
Observations	Weekly during the test period until the end of the test (day 21) regarding mortality and number of juveniles per replicate.

RESULTS AND DISCUSSION

After 21 days, 4% mortality was observed in the negative control. 12% mortality was observed at the inactivated control group and the test item group. The mean number of juveniles produced per female at each test concentration was 80 juveniles/female in the negative control, 134 juveniles/female at the test item group and 107 juvenile/female in the inactivated control.

Table 9.2.2-1 Mortality (%) of *Daphnia magna* after 21 days of exposure to Thuricide SC

	Number of daphnids introduced	Number of dead daphnids	Mortality (%)
Negative control	50	2	4
Inactivated control	50	6	12
1.00×10^6 cfu/mL	50	6	12

Table 9.2.2-2 Reproduction of *Daphnia magna* after 21 days of exposure to Thuricide SC and average juveniles per female

	Number of daphnids introduced	Total number of juveniles after 21 days	Average (juveniles per female)
Negative control	50	4018	80
Inactivated control	50	5369	107
1.00×10^6 cfu/mL	50	6711	134

CONCLUSIONS

After 21 days of exposure, the median lethal concentration of Thuricide SC (21-d LC_{50}) under the test conditions and based on nominal concentration was considered to be greater than 1.0×10^6 cfu/mL.

Table 9.2.2-3 Toxicity effects/ Infectivity / Pathogenicity of the Btk SA-11 to aquatic invertebrates

Test method	**Test species**	**Dose range Btk strain tested**	**Observations**	**Results/Endpoint**	**Reference**
21-d (semi-static)	*Daphnia magna*	Thuricide SC (SA-12)	4% mortality was observed in the negative control. 12% mortality was observed at the inactivated control group and the test item group. The mean number of juveniles produced per female at each test concentration was 80 juveniles/female in the negative control, 134 juveniles/female at the test item group and 107 juvenile/female in the inactivated control. No signs of pathogenicity or toxicity were observed. Infectivity was not investigated.	$EC_{50} > 1.0 \times 10^9$ CFU/L	KMA 9.2.2/02 Mrotzeck Masquetto, A. (2015)

Comments and conclusion RMS:

Although the study was conducted with a liquid formulation of Btk SA-12 it is considered relevant for the evaluation of the strain since the co-formulants are inert. The possible adverse effects on *Daphnia magna* were evaluated over a test period of 21 days under semi-static conditions. The study follows guideline OPPTS 885.4240 for testing of microbial active substances. After 21 days of exposure, the median lethal concentration of Thuricide SC (21-d LC_{50}) under the test conditions and based on nominal concentration was considered to be greater than 1.0×10^6 cfu/mL. 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.

Information from open literature

In addition, Eidt (1985) investigated the effect of Btk to larvae of the aquatic insects Simuliidae, Chironomidae, Trichoptera, Megaloptera, and to nymphs of the aquatic insects Ephemeroptera and Plecoptera. Test species were exposed to Btk at 4.3, 43 and 430 IU/mL over a 20- to 30-day period. Only *Simulium vittatum* (Black fly larvae) was affected, but only at the highest concentration tested where emergence of adults was low. Effects to any of the other organisms, particularly *Prosimulim fuscum*/*mixtum* (Black fly larvae), were only suggested. The lowest concentration tested was twice the worst case transitory concentration peaks expected in water following aerial forest spraying in New Brunswick, Canada at 3.0 x 10^{10} IU/ha.

Melin & Cozzi (1990) reviewed the safety to non-target invertebrates of Lepidopteran strains of *Bacillus thuringiensis* and their β-exotoxins and found that studies with freshwater microcrustaceans, mites and insects led to no Btk related toxic effects at, or more often, well above normal application rates. This was a similar conclusion made for marine invertebrates where only a brine shrimp showed signs of effects which were considered attributable to formulation particulates in the extremely high doses physically affecting the test organisms.

The response of aquatic insects to an application of *Bacillus thuringiensis* subsp. *kurstaki*, at 200 IU/mL in a forest stream was evaluated by Kreutzweiser *et al.* (1994). A slight, short-term (during the ½ hour application) increase in drifting invertebrates 10 m below the application site was considered inconsequential in terms of reduction of benthos. Sufficient data for analysis were available for 12 benthic invertebrates. No significant effects on species abundance occurred with 11 of these species. The 12th species, a stonefly, *Leuctra tenius* (Pictet) was reduced by ca. 70 % at the treated site four days after application and abundance remained lower, but not significantly, when compared to the reference site, for at least 18 days. The toxicity of Btk to *L. tenius* was thereafter investigated in the laboratory and Btk on leaf material was found not to be toxic to *L. tenius*. The reasons for the population decline in the stream environment are unknown, but if attributable to Btk, the toxicity may have been enhanced under field conditions by intrinsic environmental factors that did not occur under laboratory conditions. The application of Btk had no significant impact on the survival and growth of caged caddisfly larvae, *Pycnopsyche guttifer* (Walker) in the treated stream. The authors conclude that the limited impact of Btk on the stream invertebrate community lends support to the contention that this narrow-spectrum biological insecticide poses little risk of adverse effects on invertebrates in aquatic environments and agrees with results from previous studies. Furthermore, at normal field application rates, the level of exposure in streams will be lower than the tested concentration.

Kreutzweiser *et al.* (1992) provided further toxicity data for an array of insect taxa under natural exposure regimes and environmental conditions in recirculating laboratory bioassays and in outdoor stream channels. In the laboratory test, different invertebrate species representing twelve different taxa were exposed to *Bacillus thuringiensis* subsp. *kurstaki* in a product formulation at a concentration of 600 IU/mL. Eleven species exhibited no significant mortality after nine days. Most were < 5 % when corrected for control mortality. Mortality in one species, *Taeniopteryx nivalis*, was significantly higher than the control but showed a relatively low average mortality of only 30 %. Testing was performed at the same concentration in the outdoor examination. No significant difference in insect drift and survival of drift and benthic insects compared to a reference site occurred. The authors conclude that contamination of watersheds with Btk is unlikely to directly affect aquatic insects, even at rates well above expected environmental concentrations.

The data confirms that aquatic invertebrates will not be affected by insecticidal crystal proteins and living spores of *B. thuringiensis* subsp. *kurstaki.*

In the literature search covering the last 10 years and focussing to target possible toxicity or pathogenicity of Btk to aquatic invertebrates one article was identified, studying the susceptibility of *Daphnia similis* to microbial pest control agents including *Bacillus thuringiensis* subsp. *kurstaki.* Static acute tests revealed no acute effects on the immobility of *Daphnia similis* after 48 h (Oliveira-Filho et al, 2011).

RMS evaluation	No strain specific data for Btk SA-12 was provided for first approval, but data for the closely related Btk strain SA-11 was considered acceptable to cover the re-

	quirements. For renewal of Btk SA-12, a study is submitted assessing the side effects of Thuricide SC on *Daphnia magna*. The possible adverse effects on *Daphnia magna* were evaluated over a test period of 21 days under semi-static conditions.. After 21 days of exposure, the median lethal concentration of Thuricide SC (21-d LC_{50}) under the test conditions and based on nominal concentration was considered to be greater than 1.0×10^6 cfu/mL. No signs of treatment related pathogenicity or toxicity were observed. The endpoints can be used in risk assessment. In the literature search covering the last 10 years and focussing to target possible toxicity or pathogenicity of Btk to aquatic invertebrates one article was identified, studying the susceptibility of *Daphnia similis* to microbial pest control agents including *Bacillus thuringiensis* subsp. *kurstaki.* Static acute tests revealed no acute effects on the survival of *Daphnia similis* after 48 h (Oliveira-Filho et al, 2011). Due to available strain specific data and available knowledge about *Bacillus thuringiensis* subsp. *kurstaki* in general it can be concluded that Btk SA-12 is not toxic, pathogenic or infective to aquatic invertebrates (Table 9.2.2-3).
Endpoint: Effects on invertebrate species	$EC_{50} > 1.0 \times 10^9$ CFU/L

Cited literature

Report: KMA 9.2.2/06 – 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, published report

Ecotoxicology, 20, 1354-1360

Guideline: Not specified

GLP: No

Abstract: Microbial pest control agents or entomopathogens have been considered an interesting alternative to use instead of chemical insecticides. Knowledge of ecotoxicity data is very important to predict the hazard of any product released in the environment and subsidize the regulation of these products by governmental agencies. In the present study four new Brazilian strains of *Bacillus* and one fungus were tested to evaluate their acute toxicity to the microcrustacean *Daphnia similis*, the snail *Biomphalaria glabrata* and the dung beetle *Digitonthophagus gazella*. The microcrustaceans and the snails were exposed to entomopathogens in synthetic soft-water and the beetles were exposed directly in cattle dung. Obtained data reveal low susceptibility of the non-target species to tested microorganisms, with lethal concentrations being observed only at much higher concentrations than that effective against target insects. These results show that the tested strains are selective in their action mode and seem to be non-hazardous to non-target species.

Materials 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 (> 6, < 24 h old) per concentration were exposed to different dilutions of lyophilized 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.

Findings: 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.

Conclusions: This study pointed to the absence of acute effects on the mobility of *D. similis* exposed to two *Bacillus thuringiensis* strains in short-term tests.

Evaluation RMS	The study is not Btk SA-12 strain specific but is considered acceptable and provide supportive information confirming general absence of adverse effects of Btk on aquatic invertebrates.

B.9.2.3 Effects on algae growth

No strain specific data for Btk SA-12 was provided for first approval, but data for the closely related Btk strain SA-11 was considered acceptable to cover the requirements.

New data

A toxicity study with a formulated product based on Btk SA-12 (CoStar WG) is available demonstrating that neither Btk SA-12 nor the co-formulants in the end-use product have any effects on algae growth. The study is summarized in Document Vol. 3 MP, Section B.9, Point B.9.2.3.

Information from open literature

Koskella & Stotzky (2002) investigated the effect of the toxins of Btk on selected cultures of algae (including the species *Euglena, Chlamydomonas* and *Oedogonium*)

Mixed algal cultures were either amended or unamended with soil extract and placed in sunlight at 24 °C for 14 days. Unamended cultures consisted mostly of green algae; amended cultures consisted mostly of diatoms. Algae were exposed to various concentrations of Btk toxins for a few seconds to a few hours to either free Btk toxins or to toxins bound to an alumino-silicate clay. No bacteriostatic or bactericidal activity was detected and no inhibition of growth in pure and mixed cultures of the algae was observed at any concentration of either the bound or free Btk toxins.

The data confirms that algae will not be affected by insecticidal crystal proteins and living spores of *B. thuringiensis* subsp. *kurstaki.*

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

RMS evaluation	No strain specific data for Btk SA-12 was provided for first approval. For renewal a toxicity study with a formulated product CoStar WG (based on Btk SA-12) is available demonstrating that neither Btk SA-12 nor the co-formulants in the end-use product have any effects on algae growth. The study is summarized in Document Vol. 3 MP, Section B.9, Point B.9.2.3. The literature search covering the last 10 years and focussing to target possible toxicity or pathogenicity of Btk on algae did not provide any relevant information. Due to available strain specific data and available knowledge about *Bacillus thuringiensis* subsp. *kurstaki* in general it can be concluded that Btk SA-12 is not toxic, pathogenic or infective to aquatic plants.
Endpoint: Effects on algae	EC_{50} > 696 mg/L corresponding to 6.5×10^9 CFU/L (actual based on CFU determinations in test medium, please refer to Vol. 3 MP, Section B.9, Point B.9.2.3)

B.9.2.4 Effects on plants other than algae

No studies were submitted for the original approval of *Bacillus thuringiensis* subsp. *kurstaki* strains SA-11, SA-12 and EG2348 and no new studies have been submitted. Btk SA-12 is toxic specifically to insects of the Lepidopteran order and no effects on aquatic plants from applications of Btk SA-12 in insecticidal formulations targeted specifically at these insects is expected or envisaged. Furthermore, there has been a lack of reports of nega-

tive effects on plants from numerous studies on the persistence and fate of Btk on plants, and no reported negative effects from decades of use in agricultural and forestry environments.

Btk is a typical soil microorganism. Persistence and growth in surface water is therefore not expected.

No effect on higher aquatic or terrestrial plants is expected. Please refer to point B.9.7 where two studies on phytotoxicity is evaluated.

RMS evaluation	No substantial new information is submitted for renewal of the strain according to Regulation (EC) 1107/2009 and is not required. The literature search covering the last 10 years and focussing to target possible toxicity or pathogenicity of Btk on terrestrial plants did not provide any relevant information. Products based on Btk SA-12 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 Btk SA-12 and available knowledge about *Bacillus thuringiensis* subsp. *kurstaki* in general it can be concluded that Btk SA-12 is not toxic, pathogenic or infective to terrestrial plants.
Endpoint: Effects on aquatic plants	Not toxic, pathogenic or infective to aquatic plants based on available experience with Btk SA-12 products and efficacy testing.

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

Summary of the studies on effects on aquatic organisms treated with the Btk SA-12

Group	**Test substance**	**Time-scale**	**Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects)**
Fish species (specify): *Danio rerio*	Thuricide SC (SA-12)	30-d (semi-static)	$LC_{50} > 5.0 \times 10^9$ CFU/L
Fish species (specify): *Oncorhynchus mykiss*	CoStar WG	96-h (static)	$LC_{50} > 51$ mg/L corresponding to 4.7×10^8 CFU/L
Invertebrate species (specify): *Daphnia magna*	Thuricide SC (SA-12)	21-d (semi-static)	$EC_{50} > 1.0 \times 10^9$ CFU/L
Invertebrate species (specify): *Daphnia magna*	CoStar WG	48-h (static)	$EC_{50} > 141$ mg/L corresponding to 1.3×10^9 CFU/L
Effects on algae: (species, growth, growth rate, capacity to recover)	*Desmodesmus subspicatus* CoStar WG 72-h (static) $EC_{50} > 696$ mg/L corresponding to 6.5×10^9 CFU/L		
Effects on aquatic plants (species, growth, growth rate, capacity to recover)	Not toxic, pathogenic or infective to aquatic plants based on available experience with Btk SA-12 products and efficacy testing.		

B.9.3 Effects on bees

For the first approval of Btk SA-12 no study of effects on bees was evaluated. The approval was based on a study of the closely related strain Btk EG2348 and literature data.

New data 2016

For renewal of Btk SA-12, two studies are submitted assessing the side effects of Thuricide SC on honeybees. Although the studies were conducted with a liquid formulation of Btk SA-12 it is considered relevant for the evaluation of the strain as it follows OPPTS guidelines (since 2010 known as OCSPP guidelines) for testing of microbial active substances. More details on the formulation of the product are provided in the confidential Volume 4. Due to control mortality exceeding 20% after 96 and 120 hours, respectively, the oral and contact exposure studies were terminated after 4 and 5 days, respectively. Nevertheless, the studies demonstrate absence of adverse effects of Btk SA-12 within this time frame.

Furthermore, one study is submitted assessing the effects of different Btk strains (including SA-12) on mortality of adult bees in a field study.

In addition, it is referred to a study conducted with the formulated product Delfin WG also following OECD Guidelines 213 & 214 (1998) and OPPTS 885.4380 (1996), please refer to Vol. 3 MP, Section B.9, Point B.9.3.3. Delfin WG contains Btk SA-11 which is closely related and very similar to Btk SA-12. The study with Delfin WG demonstrates absence of adverse effects on bees over a time period of 19 and 15 days, respectively, for oral and contact exposure.

Report:	KMA 9.3/06 - Minei, C. (2015a)
Title:	Thuricide SC: Honeybee (*Apis mellifera*), acute oral toxicity test
Document No:	Report No. RL1299/2015ABO-B
Guidelines:	OPPTS 885.4380 Honey bee testing, Tier I, 1996
GLP	Yes
Validity	Yes

Executive summary

The acute oral toxicity effects of Thuricide SC on healthy worker bees (*Apis mellifera*) were evaluated over a test period of 96 hours. The test was conducted with a nominal concentration of 1.0×10^6 cfu/mL; two control groups (inactivated control at a dose level of 1.0×10^6 cfu/mL and a negative control) were also included in the test. Each test concentration included 6 replicates containing 15 bees each.

After 96 hours, 14% mortality was observed in the negative control. 7% mortality was observed at the inactivated control group and 21% mortality at the test item group. Abnormal behaviour as slow movements was observed in surviving bees in groups of the test item group and the inactivated control. Moribund bees were observed at the control group and the inactivated control.

No statistical significant toxic effect was observed in the bees at the tested item group and the inactivated control when compared with the control group (Fisher's exact test).

After 96 hours of exposure, the median lethal oral dose of Thuricide SC (96-h LC_{50}) under the test conditions and based on nominal concentration was considered greater than 1.0×10^6 cfu/mL.

MATERIAL AND METHODS

Test Item

Designation	Thuricide SC
Active ingredient	*Bacillus thuringiensis* subsp. *kurstaki* SA-12
Purity	5.0×10^9 cfu/mL (19%)
Characteristics	liquid
Batch no.	001-14-14400
Expiration date	August 2016

Test System

Species	*Apis mellifera* (worker bees)

Source	Apiário Cacilda Alves Moreira, Santana de Parnaíba, SP, Brazil
Number	15 per replicate
Food	Sugar syrup *ad libitum*
Acclimatisation	1 day at test conditions

Test Conditions

Temperature	28.1 - 30.3°C
Photoperiod	Darkness
Rel. Humidity	54 - 67%

Study Design and Methods

In-life dates	14.04.2015 to 18.04.2015
System	Acute oral toxicity
Duration	96 hours
Test vessel	600 mL beakers
Concentration	1.0×10^6 cfu/mL, inactivate control at 1.0×10^6 cfu/mL, negative control 6 replicates per test concentration and controls
Experimental treatment	Bees were exposed to 300 µL of a 50% sucrose sugar solution with test substance for 4 hours. After 4 h of exposition to the tests substance and during the study the bees were fed *ad libitum* with sugar syrup and water.
Observations	Assessment for mortality and abnormal behaviour was recorded 4, 24, 48, 72 and 96 hours after the exposure.
Statistics	Fisher's Exact Test was used to compare the mortality observed in the test substance treatment to the control.

RESULTS AND DISCUSSION

After 96 hours, 14% mortality was observed in the negative control. 7% mortality was observed at the inactivated control group and 21% mortality at the test item group. Abnormal behaviour as slow movements was observed in surviving bees in groups of the test concentration and the inactivated control. Moribund bees were observed at the control group and the inactivated control.

No statistical significant toxic effect was observed in the bees at the tested dose and the inactivated control when compared with the control group (Fisher's exact test).

Table 9.3-1 Results of the oral toxicity test with *Apis mellifera* after 96 h of exposure to Thuricide SC

	Number of bees introduced	Number of dead bees	Mortality (%)
Negative control	90	13	14
Inactivated control	90	6	7
1.00×10^6 cfu/mL	90	19	21

CONCLUSIONS

After 96 hours of exposure, the median lethal oral dose of Thuricide SC (96-h LC_{50}) under the test conditions and based on nominal concentration was considered greater than 1.0×10^6 cfu/mL.

Table B.9.3-2. Toxicity effects / Infectivity / Pathogenicity of Btk SA-12 to bees

Test species	Honey bee (*Apis mellifera*)
Toxicity	Oral: LD_{50} (4 d) > 1×10^9 CFU/L

Infectivity / Pathogenicity	Absence of pathogenicity cannot be demonstated in the 4 days' time frame. Infectivity not tested.

Comments and conclusion RMS:

For renewal of Btk SA-12, acute oral toxicity effects of Thuricide SC on healthy worker bees (*Apis mellifera*) were evaluated over a test period of 96 hours. Due to control mortality exceeding 20% after 96 hours, the study was terminated. After 96 hours, 14% mortality was observed in the negative control. 7% mortality was observed at the inactivated control group and 21% mortality at the test item group. No statistical significant toxic effect was observed in the bees at the tested dose and the inactivated control when compared with the control group. Consequently, no adverse effects directly caused by Btk SA-12 were found within the 4 days' time frame. The study is considered not sufficient to address the data requirement due to the high background mortality seen in the study. Therefore, the study results can only be used as indicative in the risk assessment.

Report:	KMA 9.3/07 - Minei, C. (2015b)
Title:	Thuricide SC: Honeybee (*Apis mellifera*), acute contact toxicity test
Document No:	Report No. RL1300/2015ABC-B
Guidelines:	OPPTS 885.4380 Honey bee testing, Tier I, 1996
GLP	Yes
Validity	Yes

Executive summary

The acute contact toxicity effects of Thuricide SC on healthy worker bees (*Apis mellifera*) were evaluated over a test period of 120 hours. The test was conducted with a nominal concentration of 1.0×10^6 cfu/mL; two control groups (inactivated control at a dose level of 1.0×10^6 cfu/mL and a negative control) were also included in the test. Each treatment group included 6 replicates containing 15 bees each.

After 120 hours, 20% mortality was observed in the negative control. 22% mortality was observed at the inactivated control group and 28% mortality at the test item group. Abnormal behaviour as slow movements was observed in surviving bees in groups of the test item group, the inactivated control and the negative control. Moribund bees were observed at the control group and the test item group.

No statistical significant toxic effect was observed in the bees at the tested dose and the inactivated control when compared with the control group (Fisher's exact test).

After 120 hours of exposure, the median lethal contact dose of Thuricide SC (120-h LC_{50}) under the test conditions and based on nominal concentration was considered greater than 1.0×10^6 cfu/mL.

MATERIAL AND METHODS

Test Item

Designation	Thuricide SC
Active ingredient	*Bacillus thuringiensis* subsp. *kurstaki* SA-12
Purity	5.0×10^9 cfu/mL (19%)
Characteristics	liquid
Batch no.	001-14-14400
Expiration date	August 2016

Test System

Species	*Apis mellifera* (worker bees)
Source	Apiário Cacilda Alves Moreira, Santana de Parnaíba, SP, Brazil
Number	15 per replicate

Food	Sugar syrup *ad libitum*
Acclimatisation	2 hours at test conditions

Test Conditions

Temperature	18.3 - 30.3°C
Photoperiod	Darkness
Rel. Humidity	57 - 78%

Study Design and Methods

In-life dates	30.03.2015 to 04.04.2015
System	Acute contact toxicity
Duration	120 hours
Test vessel	600 mL beakers
Concentration	1.0×10^6 cfu/mL, inactivate control at 1.0×10^6 cfu/mL, negative control 6 replicates per test concentration and controls
Experimental treatment	Bees were exposed to 1µL of the test solution applied on the dorsal side of the thorax of each bee with a micro piston pipette. During the study the bees were fed *ad libitum* with sugar syrup and water.
Observations	Assessment for mortality and abnormal behaviour was recorded 4, 24, 48, 72, 96 and 120 hours after the exposure.
Statistics	Fisher's Exact Test was used to compare the mortality observed in the test substance treatment to the control.

RESULTS AND DISCUSSION

After 120 hours, 20% mortality was observed in the negative control 22% mortality was observed at the inactivated control group and 28% mortality at the test item group. Abnormal behaviour as slow movements was observed in surviving bees in groups of the test item group, the inactivated control and the negative control. Moribund bees were observed at the control group and the test concentration group.

No statistically significant toxic effect was observed in the bees at the tested dose and the inactivated control when compared with the control group (Fisher's exact test).

Table 9.3-3 Results of the contact toxicity test with *Apis mellifera* after 120 h of exposure to Thuricide SC

	Number of bees introduced	Number of dead bees	Mortality (%)
Negative control	90	18	20
Inactivated control	90	20	22
1.00×10^6 cfu/mL	90	25	28

CONCLUSIONS

After 120 hours of exposure, the median lethal oral dose of Thuricide SC (120-h LC_{50}) under the test conditions and based on nominal concentration was considered greater than 1.0×10^6 cfu/mL.

Table B.9.3-4: Toxicity effects / Infectivity / Pathogenicity of Btk SA-12 to bees

Test species	Honey bee (*Apis mellifera*)
Toxicity	Contact: LD_{50} (5 d) > 1×10^9 CFU/L
Infectivity / Pathogenicity	Absence of pathogenicity cannot be demonstated in

	the 5 days' time frame. Infectivity not tested.

Comments and conclusion RMS:

For renewal of Btk SA-12, acute contact toxicity effects of Thuricide SC on adult honey bees (*Apis mellifera*) were evaluated over a test period of 120 hours. After 120 hours, 20% mortality was observed in the negative control 22% mortality was observed at the inactivated control group and 28% mortality at the test item group. Abnormal behaviour as slow movements was observed in surviving bees in groups of the test item group, the inactivated control and the negative control. Moribund bees were observed at the control group and the test concentration group. No statistical significant toxic effect was observed in the bees at the tested dose and the inactivated control when compared with the control group. The study is considered not sufficient to address the data requirement due to the high background mortality seen in the study. Therefore, the study results can only be used as indicative in the risk assessment.

Report:	KMA 9.3/08 - Mayer, D.F. (1990)
Title:	Effect of Sandoz Bt Products on adult Honeybee (*Apis mellifera* L.) mortality
Document No:	Report No. 90/01
Guidelines:	EPA Guideline number 151A-10
GLP	Yes
Validity	Not available

Executive summary

The toxicity to adult worker honeybees (*Apis mellifera*) of different strains of *Bacillus thuringiensis* subsp. *kurstaki* (including SA-12) was evaluated in a field study over a period of 24 days. The test was conducted with a nominal concentration of 10^6 cfu/mL; provided as sucrose solution with an in-hive feeder. Untreated sugar syrup was used for the control treatment. For each treatment, 4 colonies of bees in a 2-storey hive were used. The number of dead bees was assessed using a Todd dead bee entrance trap.

In the following, only the materials, methods, results and conclusion for *Bacillus thuringiensis* subsp. *kurstaki* strain SA-12 are presented.

No significant differences in the number of dead worker bees treated with Btk SA-12 compare to the control treatment were recorded (Duncan's multiple range test).

MATERIAL AND METHODS

Test Item

Designation	SA-12
Active ingredient	*Bacillus thuringiensis* subsp. *kurstaki* SA-12
Purity	1.4×10^{10} cfu/mL
Batch no.	P54-1

Test System

Species	*Apis mellifera carnica* (worker bees)
Source	Not available
Number	4 colonies of 2-storey hives per treatment
Food	Sugar syrup *ad libitum*

Study Design and Methods

In-life dates	24.08.1990 to 17.09.1990
System	Field study near Prosser, WA (USA)
Duration	24 days

Hives	2-storey hives with 10 frames in the bottom box and 9 frames in the top box in 10-frame Langstroth equipment; at each hive a Todd dead bee entrance trap was attached
Concentration	10^6 cfu/mL, negative control mixed in sucrose syrup (50% by volume)
	4 replicates per test concentration and controls
Experimental treatment	Bees were exposed to 2 L of the test solution provided by an in-hive feeder.
Observations	Assessment for mortality and abnormal behaviour was recorded daily during the test.
	The amount of sugar syrup consumed was determined at day 1 and 2 after test start.
Statistics	Duncan's multiple range test was used to compare the mortality observed in the test substance treatment to the control.

RESULTS AND DISCUSSION

No significant differences in the number of dead worker bees treated with Btk SA-12 compare to the control treatment were recorded (Duncan's multiple range test).

CONCLUSIONS

After 24 days of exposure, *Bacillus thuringiensis* subsp. *kurstaki* SA-12 demonstrated no adverse effect on *Apis mellifera* in a field study.

Table B.9.3-5: Toxicity effects / Infectivity / Pathogenicity of Btk SA-12 to bees

Test species	Honey bee (*Apis mellifera*)
Toxicity	No adverse effects observed in a field study
Infectivity / Pathogenicity	The study is not designed to demonstrate infectivity or pathogenicity.

Comments and conclusion RMS:

For renewal of Btk SA-12, toxicity to adult worker honeybees (*Apis mellifera*) of different strains of *Bacillus thuringiensis* subsp. *kurstaki* (including SA-12) was evaluated in a field study over a period of 24 days. The test was conducted with a nominal concentration of 10^6 cfu/mL; provided as sucrose solution with an in-hive feeder. Untreated sugar syrup was used for the control treatment. For each treatment, 4 colonies of bees in a 2-storey hive were used. The number of dead bees was assessed using a Todd dead bee entrance trap. No significant differences in the number of dead worker bees treated with Btk SA-12 compared to the control treatment were recorded. The study is described briefly and no agreed test guideline was followed. The result indicate That Btk SA-12 demonstrated no adverse effect on *Apis mellifera* in a field study After 24 days of exposure. The study is considered supportive.

Information from open literature

In addition, in Canada, Buckner *et al.* (1974) assessed the impact of the formulations DiPel (*Bacillus thuringiensis* subsp. *kurstaki*) on Honey bee populations during a field trial for spruce budworm control. During, and up to one week following the aerial application treatment period, Honey bee mortality, pollen collected, hive activity and hive weights were monitored, and young brood development was observed. None of the applied Btk formulations adversely affected the Honey bee colonies, even when the entire foraging area of the Honey bees was treated.

In an oral toxicity experiment with young adult honey bees, pollen-based food containing either purified and activated Cry 1 Ba δ-endotoxin (at 0.025, 0.25 and 1.0 %), or Btk formulations Dipel 2X (*Bacillus thuringiensis* subsp. *kurstaki* Strain HD-1 formulation) (at 0.25 and 1.0 % w/w of a.s. in pollen food) or Foray 48B (at 0.25 % w/w of a.s. in pollen food) was fed to bees for 7 days, (Malone *et al.*, 1999). Bee survival was monitored during this period after which feed was then replaced with untreated pollen-based food and the survival of bees was

monitored for up to 70 days or until all test bees were dead. Bee survival time was unaffected and the rate of food consumption was the same as for control groups for all treatments except the Dipel 2X 1.0 % treatment group. Here, survival and food consumption were significantly lower than in the control. Following exposure, when bees were receiving untreated feed, food consumption in this group was observed to be significantly higher than in other groups, indicating no harmful effect to surviving bees. It should be noted that the rate of 0.25 % w/w of active substance (a.s.) in pollen food approximates the minimum LD_{50} for a pesticide which is virtually non-toxic to honey bees. And a rate of 1.0 % w/w of a.s. in pollen food is equivalent to 4 % of total protein, an unrealistically high dose which was used to allow direct comparison with the high dose Cry 1 Ba treatment.

Cantwell *et al.* (1972) summarised research into the toxicity of various bacteria to honey bees. They concluded that Bt complexes and products based on Bt which are free from exotoxins (as is the case with *Bacillus thuringiensis* subsp. *kurstaki* Strain SA-11, SA-12 and EG2348) cause no harm to honey bees when tested under laboratory, simulated field and field conditions. Lehnert & Cantwell (1978) reiterate these findings and draw the same conclusion. Krieg (1973) determined that only the exotoxins from Bt complexes caused harmful effects and mortality to honey bees in the laboratory.

In the literature search covering the last 10 years and focussing to target possible toxicity or pathogenicity of Btk to honeybees and bumblebees two articles were identified, studying the susceptibility of *Apis mellifera* and *Bombus terrestris* to microbial pest control agents including *Bacillus thuringiensis* subsp. *kurstaki* (del Mar Leza et al, 2014 and Mommaerts et al, 2009). Both studies confirm that Btk has no toxic or pathogenic effects in honeybees and bumblebees.

RMS evaluation and conclusion	For renewal of Btk SA-12, two studies are submitted assessing the side effects of oral and contact exposure by Thuricide SC on honeybees. Due to control mortality exceeding 20% after 96 and 120 hours, respectively, the studies were terminated after 4 and 5 days, respectively. Nevertheless, the studies demonstrate absence of adverse effects of Btk SA-12 within these time frames. However, the studies are considered not sufficient to address the data requirement due to the high background mortality seen in the studies. Therefore, the study results can only be used as indicative in the risk assessment. Furthermore, one study assessing the effects of different Btk strains (including SA-12) on mortality of adult bees in a field study indicate no adverse effects. In addition, it is referred to a study conducted with the formulated product Delfin WG also following OECD Guidelines 213 & 214 (1998) and OPPTS 885.4380 (1996), please refer to Volume 3 MPCP, section B.9.3.1. Since the product Delfin WG only contains the micro-organisms and an inert co-formulant studies performed on the product is considered applicable also to cover data for the active substance. Delfin WG contains Btk SA-11 which is closely related and very similar to Btk SA-12. According to the EFSA peer review of the risk assessment of the five Btk strains[2] the extrapolation between different *Bacillus thuringiensis kurstaki* strains can be considered acceptable for non-target organisms, except for daphnids and non-target arthropods. Therefore, the study on Delfin WG is considered relevant with regard to the evaluation of effects on bees of Btk SA-12. The study with Delfin WG demonstrates absence of adverse effects over a time period of 19 and 15 days, respectively, for oral and contact exposure. Endpoint are: Oral: LD_{50} (19 d) > 82 µg product/bee or > 4.2×10^6 CFU/bee Contact: LD_{50} (15 d) > 100 µg product/bee or > 5.1×10^6 CFU/bee Due to available strain specific data, information on closely rated strains and available knowledge about *Bacillus thuringiensis* subsp. *kurstaki* in general it can be concluded that Btk SA-12 is not toxic or pathogenic in honeybees or bumblebees.
Endpoint: Effects on bees	<u>Btk SA-12</u> Contact: LD_{50} (5 d) > 1×10^9 CFU/L Oral: LD_{50} (4 d) > 1×10^9 CFU/L <u>Btk SA-11</u> Oral: LD_{50} (19 d) > 82 µg product/bee or > 4.2×10^6 CFU/bee

[2] European Food Safety Authority: Conclusion on the peer review of the pesticide risk assessment of the active substance *Bacillus thuringiensis* subsp. *kurstaki* (strains ABTS 351, PB 54, SA 11, SA 12, EG 2348). EFSA Journal 2012;10(2):2540

	Contact: LD_{50} (15 d) > 100 µg product/bee or > 5.1×10^6 CFU/bee

Cited literature

Report: KMA 9.3/09 – 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)

Spanish Journal of Agricultural Research, 12(2), 405-408

Guideline: Not specified

GLP: No

Abstract: Honeybee populations around the world are experiencing a decrease in colony numbers probably due to a combination of different causes, such as diseases, poor nutrition and frequent applications of insecticides to control pests. Previous studies about the effect of pesticide *Bacillus thuringiensis* subsp. *kurstaki* (Btk) on *Apis mellifera* L. report different results. 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. To achieve it, the brood cell surface was photographed in every sampling, and data were analysed using a method based on image treatment software. A total of 480 pictures were examined from two groups of four nucleus hives in two areas, one receiving aerial spraying with Btk and the other without treatment. A mixed factorial design was realized to analyse the data showing no differences in colony performance between the two groups of colonies either before the treatment, during and at the end of the assay. Furthermore, the brood surface ratio of Btk treated/ untreated increased along 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.

Materials and Methods: 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.

Findings: The percentage of brood in both groups of hives shoed a strong parallelism throughout the experiment. No significant differences between groups were found. During the first three samplings the brood were 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 ration increased throughout the experiment.

Conclusions: The results suggest that Btk do not affect the brood development of honeybees.

Evaluation RMS	In del Mar Leza et al., 2014a product containing 11.8 x 10^6 IU/g *Bacillus thuringiensis* subsp. *kurstaki* (Btk) was sprayed at 3.5 L/ha. The resulting load of IU/ha would be 4.13 x 10^{10} IU/ha. This is only a little lower than the range for the intended field uses of CoStar WG (1.35 – 1.8 x 10^{11} IU/ha). From the information in the literature reference the application rate is not 100% clear. The study is not Btk SA-

	12 strain specific but is considered acceptable and provide supportive information confirming general absence of adverse effects of Btk on bees.

Report: KMA 9.3/10 – 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

Guideline: Not specified

GLP: No

Abstract

BACKGROUND: *Bacillus thuringiensis* (Bt) and its protein crystals are used worldwide, either as a spray or when expressed in transgenic crops, for the control of pest insects. However, owing to their intensive use, there exists a debate regarding the involvement of this microbial insecticide in bee colony losses. In this study, in a tiered approach using laboratory microcolonies, an evaluation was made of the potential lethal and sublethal hazards on colony reproduction and foraging behaviour of workers of the bumblebee *Bombus terrestris* (L.) of two commercial Bt strains: *kurstaki* (Dipel®) and *aizawai* (Xentari®). Bumblebees, like honeybees, are intensively used in modern agriculture for pollination and fulfil a crucial role in the natural ecosystem.

RESULTS: Exposure of bumblebees dermally or via treated pollen to either of the Bt formulation at the field recommended rate (0.1%) caused no reduction in survival. With respect to reproductive effects, *kurstaki* was harmless. In addition, *kurstaki* at 0.1% in the feeding sugar water did not impair the foraging behaviour, resulting in normal nest colony performance.

CONCLUSION: The results with *kurstaki* demonstrated that, in general, the Bt strains are safe to *B. terrestris*. In addition, the authors believe that to draw firm conclusions regarding the hazards of Bt to bumblebees would require more information on relevant concentrations of Bt products in the environment. Hence, routine testing for lethal and sublethal effects is recommended to ascertain combined use of Bt products and bumblebees in modern agriculture.

Materials and Methods: The study was conducted in the laboratory. In order to determine the effects of Dipel® (1.6×10^4 IU/mg), the workers were exposed via three routes: contact by dermal application, orally via feeding treated sugar water and treated pollen. For each treatment, four nests were tested, each with five workers. 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 MFRC (field recommended concentration). The mortality of the worker bees was assessed weekly during the 11 weeks of exposure. The effects on reproduction were assessed by weekly counting the number of male offspring produced per nest. In order to assess the sub-lethal effect on the foraging capacity, two artificial nest boxes were connected 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 paced in the second box.

The experiment with Bt subsp. *aizawai* of the study is not presented here.

Findings: Dipel® at its MFRC of 0.1% resulted in no acute worker mortality via contact exposure or when delivered orally vial treated sugar water and treated pollen. No negative effects on reproduction 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 were observed. The survival of workers after 11 weeks was not affected.

Conclusions: The results with Bt subsp. *kurstaki* demonstrated that, in general, the Bt strains are safe to *B. terrestris* bumblebees.

Evaluation RMS	In Mommaerts et al. (2010) the Btk product Dipel (containing Btk strain ABTS-351) was used at a rate of 1.6 x 10^4 IU/mL. The max rate of CoStar WG in the spraying solution is 0.3% corresponding to 2.7×10^5 IU/mL. Though higher than the endpoint from the literature reference, the dose is in the same range. It is noted that the exposure time in the study is completely unrealistic (11 weeks of exposure at the MFRC). Apart from the exposure period, such exposure to the undiluted

	MFRC in general is rather unrealistic. Bumblebees will surely escape from fields during spraying campaigns so that topical exposure will not occur et al. Also, exposure to undiluted MFRC via drinking water or pollen is a strong overestimate of the actual possible exposure. The study is not Btk SA-12 strain specific and the exposure is unrealistic. Therefore should the study results only be used as supportive information confirming the absence of adverse effects of Btk on bumble bees.

B.9.4 Effects on arthropods other than bees

No strain specific data for Btk SA-12 was provided for first approval, but data for closely related Btk strains was considered acceptable to cover the requirements. Therefore, this summary of effects on terrestrial arthropods other than bees from the (DAR 2008) is provided.

Two studies assess the effect of exposure of the indicator non-target arthropod species *Aphidius rhopalosiphi* and *Typhlodromus pyri* to applications of Delfin WG. Only slight statistically significant effects on mortality (respectively 12.5 and 13 %) were observed in worst case laboratory tests with *A. rhopalosiphi* and *T. pyri* at the tested rate of 4.5 kg Delfin WG/ha. Significant adverse effects on reproduction did not occur in either species.

The effect of *Bacillus thuringiensis* strain EG2348 on the parasitic hymenoptera, *Brachymeria intermedia* following topical application showed a low mortality of 15 % at the tested concentration of 0.56 mg/*Brachymeria intermedia* 48 hours following application. In similar experiments with the same test substance at the same concentration rate, *Chrysoperla rufilabris* were completely unaffected while a low mortality of 5 % in Ladybird beetle occurred. *Chrysoperla carnea* larvae were also exposed to various *Bacillus thuringiensis* powders, including EG2348 for 48 hours at 2.24 kg test substance/ha. No statistically significant mortality compared to the control groups occurred in any of the test substance treatment groups. Mortality of just 4 % occurred in the EG2348 treatment group. However, the results of the topical application of *Bacillus thuringiensis* strain EG2348 are difficult to evaluate because the application was conducted in acetone, which might affect the spores and the delta-toxins. Results of all available studies are summarised in Table 9.4-1.

Table 9.4-1 Summary of results from toxicity studies with terrestrial arthropods other than bees

Test species	Test method	Application rate / Test substance	Effects	Reference (DAR 2008)
Aphidius rhopalosiphi	Laboratory limit test, 48-hour mortality, 13-day reproduction	4.5 kg/ha Delfin WG (a.s. Btk SA-11)	LR_{50} > 4.5 kg Delfin WG/ha (> 2.74 x 10^{11} IU/ha)	IIIM 10.4/01 [a] Warmers, 2005a
Typhlodromus pyri	Laboratory limit test, 7-day mortality, 14-day reproduction	4.5 kg/ha Delfin WG (a.s. Btk SA-11)	LR_{50} > 4.5 kg Delfin WG/ha (> 2.74 x 10^{11} IU/ha)	IIIM 10.4/02 [a] Warmers, 2005b
Brachymeria intermedia	48 hours, laboratory limit test, contact	*0.56 mg Bacillus thuringiensis kurstaki* EG2348	LR_{50} > 0.56 mg Btk/ *Brachymeria intermedia*	IIM 8.8/01 Hoxter et al, 1987a
Chrysoperla rufilabris larvae	48 hours, laboratory limit test, contact	*0.56 mg Bacillus thuringiensis kurstaki* EG2348	LR_{50} > 0.56 mg Btk/ Green lacewing larvae	IIM 8.8/02 Hinken & Jaber, 1987
Chrysoperla carnea larvae	96 hours, Bell Jar dusting test, contact, residue & oral	*2.24 kg Btk/ha Bacillus thuringiensis kurstaki* EG2348	LR_{50} > 2.24 kg Btk/ha	IIM 8.8/03 Kirkland, 1988b
Ladybird beetles	48 hours, laboratory limit test, contact	*0.56 mg Bacillus thuringiensis kurstaki* EG2348	LR_{50} > 0.56 mg Btk/ Ladybird beetle	IIM 8.8/04 Hoxter et al, 1987b

New data 2016

It is also referred to two studies assessing toxic effects with the end-use product CoStar WG on *Typhlodromus pyri* and *Aphidius rhopalosiphi*. Please refer to Vol. 3 MP, Section B.9, Point B.9.4. For *Typhlodromus pyri*, a statistically significant effect on mortality was observed at 12000 g product/ha; however, the effect was well below the trigger value of 50%. No inhibitory effect on reproduction of *T. pyri* was observed at 12000 g prod-

uct/ha. No effects on survival and reproduction (parasitisation rate) of *A. rhopalosiphi* were observed at 12000 g product/ha.

Information from open literature

Melin & Cozzi (1990) reviewed the safety to non-target invertebrates of Lepidopteran strains of *Bacillus thuringiensis* and their β-exotoxins. The exposure time on leaves is considered to be short with degradation of spores, cells and crystals resulting from exposure to solar radiation. Insects of the orders *Orthoptera* (Chinese praying mantid) and *Dermaptera* (striped earwig) were unaffected by exposure to Btk either by ingestion of infected larval cabbage loopers or exposure in soil at rates exceeding normal application rates, respectively. Similarly, studies on various species of the orders *Heteroptera* (e.g. *Jalysus spinosus*, *Nabis* spp., *Geocoris* spp.), Coleoptera (e.g. *Bembidion lampros*, *Stethorus punctum*, *Pinacodera platicollis*) and Diptera and on the lacewing, *Chrysoperla carnea*, showed no toxicologically detrimental effects following exposure to formulations containing Btk. Species of the order *Hymenoptera* were extensively researched and, for the majority of reports, no detrimental effects from oral and contact exposure to varied amounts of Btk were observed. Some insects were thought to be able to distinguish between host/prey insects infected with Btk and avoided utilizing or feeding on these organisms. In some cases, host insects infected with Btk and parasitized by other invertebrates saw a prolonged development time of the parasite within the host and/or a reduced emergence rate or reduced size of the emerged parasitic adult. This must be regarded as a secondary knock-on effect of the insecticide while it actively works to reduce pest numbers. On the other hand, the effect of Btk in reducing feeding rates in some host insects may lead to an increase in parasitism rates, as the hosts remain at sizes suitable for parasitism for longer periods.

In Canada, Buckner *et al.* (1974) assessed the impact of a DiPel formulation (*Bacillus thuringiensis kurstaki)* on non-target insect populations from the forest floor to foliage dwelling species during a field trial for spruce budworm control. Six and thirty days following the aerial application treatment period, non-target insect populations were monitored. None of the applied Btk formulations significantly adversely affected non-target insect populations in the treated areas compared to control areas.

Hamed (1979) assessed the impact of an oral intake over six days of a 1:1 mixture of honey and Btk from several commercial preparations, to seven species of parasites and one species of predator of the Bird cherry ermine moth *Yponomeuta evonymellus* (*Lep., Yponomeutidae*). Following ingestion over six days of 5 x 10^8 spores/mL and associated crystals in food sap by test insects, two tachnids (*Bessa fugax* (Rond.) and *Zenillia dolosa* (Meig.)), one ichneumonid (*Trichionotus* sp.) and the predatory shieldbug (*Picromerus bidens*) were not affected while four hymenopter species (*Diadegma armillata* (Grav.), *Pimpla turionella* L., *Ageniaspis fusicollis* Dalm. and *Tetrastichus evonymellae* (Bouché)) showed negative effects on mortality and reproduction and the presence of vegetative cells in the bodies of dead parasites. However, such a long-term exposure at this tested concentration to insects in the field following GAP application of DiPel WG will not occur when degradation of Btk is taken into account. Furthermore, since Btk was mixed with honey, the non-target organisms may have been exposed also to vegetative cells.

The data confirms that it is unlikely that non-target arthropods will be affected by insecticidal crystal proteins and living spores of *B. thuringiensis kurstaki* SA-11, SA-12 and EG2348. However predators might be affected by vegetative cells. The Rapporteur assess that such an exposure is very limited and is considered negligible.

In the literature search covering the last 10 years and focussing to target possible toxicity or pathogenicity of Btk on terrestrial non-target arthropods (other than bees) several articles were identified, studying the susceptibility of non-target arthropods to microbial pest control agents including *Bacillus thuringiensis* subsp. *kurstaki* (see below). Laboratory bioassays evaluating the mortality and fecundity of the predatory mite *Euseius victoriensis* exposed to soybean leaves treated with *Bacillus thuringiensis* subsp. *kurstaki* revealed no negative effects on mortality and fecundity (Bernard et al, 2010). Carvalho et al, 2010, classified *Bacillus thuringiensis* subsp. *kurstaki* as harmless to *Trichogramma pretiosum* in a laboratory test. Tests with cold-stored diapausing preimaginal *Trichogramma cordubensis* revealed little or no adverse effect of *Bacillus thuringiensis* subsp. *kurstaki* on emergence rates, longevity and fecundity (Garcia et al, 2008). Garantonakis and co-workers (2016) compared the selectivity of pesticides used in greenhouses on the aphid parasitoid *Aphidius colemani* and classified *Bacillus thuringiensis* subsp. *kurstaki* as harmless according to the IOBC TC. The evaluation on persistence and relative toxicity of pest control products to adults of *Trichogramma* sp. nr. *mwanzai* and *Trichogramma* sp. nr. *lutea* revealed no significant persistence of *Bacillus thuringiensis* subsp. *kurstaki* to both of the species (Momanyi et al, 2012). The side-effects of *Bacillus thuringiensis* subsp. *kurstaki* on the parasitic wasp *Trichogramma chilonis* was evaluated by Amichot et al (2016). No effect on host feeding behaviour, fecundity and parasitism success was observed. However, an effect on mortality when feeding a higher amount of spores and an extended longevity when feeding low spore doses to adult *T. chilonis* was observed.

RMS evaluation	For first approval of Btk SA-11, Btk SA-12 and Btk EG2348 two studies were submitted in which the formulated product Delfin WG was tested. The test species for these two studies were: the aphid parasitoid hymenopteras *Aphidius rhopalosiphi* and the predatory mite *Typhlodromus pyri*. Please refer to Volume 3 MPCP, section B.9.4. Furthermore, a total of 4 studies (evaluated in the DAR 2008) were submitted in which other Btk strains have been tested on the following test species: the aphid parasitoid hymenoptera, *Brachymeria intermedia*; two studies on green lacewing larvae (*Chrysoperla rufilabris* and *C. carnea*) and the Ladybird Beetle (*Hippodamia convergens*). Significant adverse effects on any of the terrestrial arthropods did not occur in any of the studies. Six new articles were identified from the literature search, studying the susceptibility of non-target arthropods to microbial pest control agents including *Bacillus thuringiensis* subsp. *kurstaki*. The studies are considered providing supportive information confirming absence of side effects of Btk on non-target arthropods. It is also referred to two studies assessing toxic effects with the end-use product CoStar WG on *Typhlodromus pyri* and *Aphidius rhopalosiphi*. The ingredients of the preparation CoStar WG are inert and no hazards to arthropods are expected. Therefore, the studies on CoStar WG are considered applicable and relevant with regard to the evaluation of effects on terrestrial arthropods of Btk SA-12. Due to available strain specific data and available knowledge about *Bacillus thuringiensis* subsp. *kurstaki* in general it can be concluded that Btk SA-12 is not toxic, pathogenic or infective to arthropods other than the target pests.
Endpoint: Effects on terrestrial arthropods other than bees	*Aphidius rhopalosiphi*: EC_{50} > 12 kg product/ha corresponding to 1.1×10^{12} IU/ha *Typhlodromus pyri*: EC_{50} > 12 kg product/ha corresponding to 1.1×10^{12} IU/ha

Cited literature

Report: KMA 9.4/04 – 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)

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

Guideline: Not specified

GLP: No

Abstract: 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. Zero- to 48-h-old juveniles, their initial food, and water supply were sprayed to runoff with a Potter tower; spinosad and wettable sulfur residues also were tested. Tests were standardized to deliver a pesticide dose comparable with commercial application of highest label rates at 1,000 liter/ha. Cumulative mortality was assessed 48 h, 4 d, and 7 d after spraying. Fecundity was assessed for 7 d from start of oviposition. No significant mortality or fecundity effects were detected for the following compounds at single-use application at 1,000 liter/ha: azoxystrobin, *Bacillus thuringiensis* (Bt) subsp. *kurstaki*, captan, chlorothalonil, copper hydroxide, fenarimol, glyphosate, hexaconazole, indoxacarb, metalaxyl/copper hydroxide, myclobutanil, nonyl phenol ethylene oxide, phosphorous acid, potassium bicarbonate, pyraclostrobin, quinoxyfen, spiroxamine, synthetic latex, tebufenozide, triadimenol, and trifloxystrobin. Iprodione and penconazole had some detrimental effect on fecundity. Canola oil as acaricide (2 liter/100 liter) and wettable sulfur (200 g/100 liter) had some detrimental effect on survival and fecundity and cyprodinil/fludioxonil on survivor. The following compounds were highly toxic (high 48-h mortality): benomyl, carbendazim, emamectin benzoate, mancozeb, spinosad (direct overspray and residue), wettable sulfur (≥400 g/100 liter), and pyrimethanil; pyrimethanil had no significant effect on fecundity of surviving females. Indoxacarb safety to *E. victoriensis* contrasts with its toxicity to key para-

sitoids and chrysopid predators. Potential impact of findings is discussed.

Materials and Methods:	The study was conducted in the laboratory. Seven assays with 32 compounds were tested; one was a commercial microbial product of *Bacillus thuringiensis* subsp. *kurstaki* (Btk) (Delfin WG). 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 assess*ed* for 7 d from start of oviposition. Other experiments of the study are not presented here.
Findings:	*Bacillus thuringiensis* subsp. *kurstaki* had no significant effects on morality or fecundity of *Euseius victoriensis* compared with the control.
Conclusions:	No negative effect on mortality and fecundity of *E. victoriensis* after exposure to *Bacillus thuringiensis* subsp. *kurstaki* could be observed.

Evaluation RMS	The study was conducted with Delfin WG (containing Btk SA-11) among other compounds. The rate tested was 100 g/100L. Mortality and fecundity have been assessed. No mortality and no effect on reproduction have been observed. Obtained endpoint would be EC > 100 g/100 L or 1 g/L. Max concentration according to renewal GAP is 2 g/L. Compared to the available *Typhlodromus* study (glass plate test), degree of exposure was much stronger (overspray till runoff). The study is considered acceptable but is not strain specific.

Report: KMA 9.4/05 – 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), Pirimor (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.

Evaluation RMS	DiPel (containing Btk strain ABTS-351) was used at a rate of 0.32 g/L water. The study is a conference paper and as such did not went through a peer review process.

	Endpoints of such studies are usually not included in the LoEP. Also, the endpoint is not strain specific. Tested rates cannot be compared to possible exposure to Btk SA-12 as the endpoints are not given in IU or CFU. The reference is considered as providing supportive information confirming absence of side effects of Btk on non-target arthropods.

Report: KMA 9.4/06 – Garcia, P.V., Pereira, N., Oliveira, L.M. (2008)

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.

Evaluation RMS	Dipel (containing Btk strain ABTS-351) was tested. There had been no effects on

	adult emergence and fecundity of *Trichogramma*. The study is not strain specific and the tested rate is not clear. The results are therefore only considered supportive information confirming absence of side effects of Btk on non-target arthropods.

Report: KMA 9.4/07 – 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 sub-lethal effects assessment. To evaluate the sub-lethal 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.

Evaluation RMS	In the study it is stated that XenTari is the tested biopesticide containing *Bacillus thuringiensis* var. *kurstaki*. However, to the best of our knowledge there exists no such biopesticide. On the other hand, XenTari (containing Bta) is a well-known biopesticide. Regardless, it is most likely a biopesticide based on Bt that was tested at 30 mg a.i./mL. Contact, residual toxicity and sublethal effects on fecundity have been assessed. The product was shown to be harmless. The study is not strain specific. Tested rates cannot be compared to possible exposure to Btk SA-12 as the endpoints are not given in IU or CFU. The reference is considered providing sup-

	portive information confirming absence of side effects of Bt on non-target arthropods.

Report: KMA 9.4/08 – 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 LC50 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.

Evaluation RMS	DiPel (containing Btk strain ABTS-351) was tested at 1 g/L. Considering the specification of 32000 IU/mg this corresponds to 3.2 x 10^7 IU/L. The max concentration

	of Btk SA-12 in the spraying solution is 2.7×10^8 IU/L. One can say that the rates are in a comparable range. Dipel was found to be harmless and had no persistent toxicity on *Trichogramma*. The obtained data are not strain specific. Therefore, the reference is considered providing supportive information confirming absence of side effects of Btk on non-target arthropods.

Report: KMA 9.4/09 – 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 and Pollution Research, 23(4), 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.

Evaluation RMS	DiPel (containing Btk strain ABTS-351) ($5x10^4$ CFU/µg) was supplied to *Trichogramma* orally to at rates of 4, 10, 20, 40, 80 and 200 µg/µL in acute toxicity assessment tests. For testing sublethal effects on longevity, host-feeding behaviour, fecundity and patch time allocation the product was administered at a rate of 4 µg/µL. The obtained LC_{50} and LC_{10} for mortality were 84.2 and 22 µg/µL corresponding to 4.2 or 1.1 $x10^6$ CFU/µL or 4.2 x 10^{12} CFU/L. Longevity was positively affected at 4 µg/µL. No effects on fecundity and parasitism success have been noted. The maximum concentration of CoStar WG in the spraying solution is 0.3% corresponding to a CFU density of Btk SA-12 of 1.7×10^{11} CFU/L (considering the maximum CFU content of 5.7 x 10^{13} CFU/kg in CoStar WG). This is much lower than the endpoints obtained for the Dipel strain. However, the results are not strain specific and should only be considered supportive information confirming the absence of adverse effects of Btk on *Trichogramma*.

B.9.5 Effects on earthworms

For first approval of Btk SA-11 study were submitted in which the formulated product Delfin WG was tested. Please refer to Volume 3 MPCP, section B.9.5.1. Based on this study it was concluded that all Btk strains under evaluation do not have any adverse effects on earthworms. No new studies are provided for renewal

Information from open literature

Information from open literature was also evaluated in the DAR 2008 of SA-11, SA-12 and EG2348 and all these data confirms that earthworms will not be affected by insecticidal crystal proteins and living spores of *B. thuringiensis kurstaki*.

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

Earthworm immunity has extensively been studied and earthworms have served as an important experimental model for immunologic research. Earthworms have evolved effective innate defence mechanisms for survival in often hostile habitats, and have experienced a long time of co-evolution with micro-organisms in their environment (Bilej *et al.*, 2010). Earthworms are able to cope with soilborne micro-organisms without being infected or negatively affected due to the long-time evolutionary co-existence (SANCO/12117/2012)[3]. Only few studies indicated pathogenic effects to earthworms, however the observed pathogenic effects of *Bacillus thuringiensis* subsp. *thuringiensis* in a prolonged study with *Lumbricus terrestris* were not attributed to the tested mBCA (Smirnoff & Heimpel, 1961; cited in SANCO/12117/2012). Addison & Holmes (1996, cited in SANCO/12117/2012) observed detrimental impacts of Bt-formulations on earthworms and other non-target soil organisms, but found no effect of unformulated and aqueous Btk at 1000 times the field concentration. The presented literature references were obtained in a general search for Btk and not by the search according to EFSA guidance and focussing on the risk assessment.

RMS evaluation and conclusion	For first approval a study was submitted in which the formulated product Delfin WG containing the closely related strain Btk SA-11 was tested. Based on this study it was concluded that Btk SA-11 as well as all Btk strains under evaluation do not have any adverse effects on earthworms. Data provided for first approval are considered acceptable to cover current requirements and no new studies are required. No substantial new information is submitted for renewal of the strain according to Regulation (EC) 1107/2009. Endpoint (effects on earthworms): *Eisenia fetida*: No signs of infectivity or pathogenicity at 1000 mg Delfin WG/kg soil (dw). Delfin WG contains Btk SA-11, which is closely related and very similar to Btk SA-12 No reports exist on any adverse effects of *Btk* to terrestrial invertebrates, and the acute toxicity study on earthworms confirmed the absence of any adverse effects of the bacterium on terrestrial invertebrates. Due to available data on closely related strains and available knowledge about *Bacillus thuringiensis* subsp. *kurstaki* in general it can be concluded that Btk SA-12 is not toxic, pathogenic or infective to earthworms.
Endpoint: Effects on earthworms	Due to available data on a closely related strain and available knowledge about *Bacillus thuringiensis* subsp. *kurstaki* in general it can be concluded that Btk SA-12 is not toxic, pathogenic or infective to earthworms.

Cited literature abstracts:

Report KMA 9.5/01 - Bilej, M., Procháuková, P., Šilerová, M., Josková, R. (2010) Earthworm Immunity

[3] 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.

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.

Evaluation RMS	No remarks

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

For first approval based on a study with the formulated product Delfin WG, containing Btk SA-11, it was concluded that Btk SA-11 as well as all Btk strains under evaluation do not have any adverse effects on soil microbial communities. Data provided for first approval are considered acceptable to cover current requirements no new studies are submitted. No substantial new information is submitted for renewal of the strain according to Regulation (EC) 1107/2009.

Information from open literature

In the literature search covering the last 10 years and focussing to target possible toxicity or pathogenicity of Btk on non-target soil micro-organisms one article was identified, studying the analysis of non-target impacts of *Bacillus thuringiensis* subsp. *kurstaki* on soil micro-organisms (O'Callaghan et al, 2007). DNA-fingerprinting of soil treated with different doses of *Bacillus thuringiensis* subsp. *kurstaki*, showed that Btk applied at 1000 × field rate caused only transient effect on the bacteria functional diversity and the total number of culturable bacteria and fungi; no effect on the diversity of predominant eubacterial populations present in soil were determined. A full study summary is provided below.

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.

- 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.

[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.

RMS evaluation	Data on Delfin WG provided for first approval are considered acceptable to cover current requirements no new studies are required. No substantial new information is submitted for renewal of the strain according to Regulation (EC) 1107/2009. Endpoint (Effects on soil microorganisms): Delfin WG has no significant effects on the nitrogen turnover and short-term respiration activity of soil microflora at tested concentrations of up to 20.0 mg/kg soil (dw), equivalent to 15 kg Delfin WG/ha. Delfin WG contains Btk SA-11, which is closely related and very similar to Btk SA-12. Although no strain specific data have been provided it was concluded that registered Btk strains do not exhibit adverse effects in soil micro-organisms. For renewal it is acceptable to conclude: Due to available data on a closely related strain and available knowledge about *Bacillus thuringiensis* subsp. *kurstaki* in general it can be concluded that Btk SA-12 is not toxic, pathogenic or infective to soil micro-organisms.
Endpoint: Effects on soil micro-organisms	Due to available data on a closely related strain and available knowledge about *Bacillus thuringiensis* subsp. *kurstaki* in general it can be concluded that Btk SA-12 is not toxic, pathogenic or infective to soil micro-organisms.

Cited literature abstracts:

Report: KMA 9.6/01 – 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.

Evaluation RMS	Foray 48B (containing Btk strain ABTS-351) was tested at very high rates (1000 × FR) and had no effect on diversity of predominant eubacterial populations present in soil. The obtained data are not strain specific. Therefore, the reference is considered providing supportive information confirming absence of side effects of Btk on non-target soil micro-organisms.

Report KMA 9.6/02 - 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.

Evaluation RMS	The study is 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.

B.9.7 Additional studies

Bacillus thuringiensis subsp. *kurstaki*, strain SA-11, SA-12 and EG2348 is toxic specifically to insects of the *Lepidopteran* order and no effects on aquatic plants from applications of Btk in insecticidal formulations targeted specifically at these insects is expected or envisaged. Furthermore, the lack of reports of negative effects on plants from numerous studies on the persistence and fate of Btk on plants, as well as lack of reported negative effects from decades of use in agricultural and forestry environments indicates that no adverse effect occurs on plants. However, the notifier has submitted two studies on the effects of Btk on seedling emergence and vegetative vigour.

KMA 9.7/01: Nontarget phytotoxicity test: Seed germination/seedling emergence, Tier 1. (Cañez, V.M., 1988a)

Guidelines: FIFRA 122-1

GLP: yes

Materials and methods:

Test substance: B. thuringiensis kurstaki EG2348 fomulated as Condor OF – a non-aqueous flowable concentrate.

Content of a.i.: Not stated

Test species: Six dicotyledon species and four monocotyledon plant species: Soybean (*Glycine max*), Lettuce (*Lactuca sativa*), Carrot(*Daucus carota*), Tomato (*Lycopersicon esculentum*), Cucumber

(*Cucumis sativus*), Cabbage(*Brassica oleracea*), Oat (*Avena sativa*), Perennial Ryegrass (*Lolium perenne*), Corn (maize)(*Zea mays*) and Onion (*Allium cepa*).

Number of organisms, size: Ten seeds of each plant species with five replicates for seed germination as well as for the seedling emergence studies.

Applied and measured concentrations: Not specifically stated. In the seed germination studies, 7 mL of Condor OF was applied to a glass petri dish containing filter paper. In the seedling emergence studies, Condor OF was applied once onto bare, sterilized soil at an application rate corresponding to 74.9 L Condor OF/ha (equivalent to 348 g a.s./ha).

Test conditions: In the seed germination studies the petri dishes were sealed to prevent moisture loss and incubated in the dark for five (cabbage, cucumber and oat) to seven days (remaining crops) at 25 ± 1 °C, or at 18 ± 1 °C for five days for lettuce.

In the seedling emergence studies plastic test pots were kept in a greeenhouse under natural light conditions which averaged 14.3 hours light per day at a mean intensity of 16,000 to 23,500 lux and watered from the top via regular irrigation which was adjusted as necessary. The duration of the studies were 21 days. The test soil was a sandy loam soil.

Findings:

No statistically significant effect of the test substance to radicle length or percent germination occurred. Effect on radicle length ranged from a 12 % increase in tomato to a 6 % percent decrease in oat.

Effect on germination ranged from an 8 % increase in carrot to a 9 % decrease in cabbage. Apart from cabbage at day 21, no statistically significant phytotoxic effects occurred ($p \leq 0.05$). Cabbage had a mean phytotoxicity rating of 0.3 in the treated group compared to 0.0 in the control group. This was, however still a very low effect. Emergence of seedlings from treated soil was only statistically significantly decreased ($p \leq 0.05$) compared to the control in corn (maize) which exhibited a 16 % decrease in emergence. The percent effect in emergence ranged from a 4 % increase in tomato to an 18 % decrease in lettuce. Plant height was not statistically significantly affected in any species. The percent effect ranged from a 10 % increase in lettuce to a 3 % decrease in ryegrass. Dry weight in cabbage was statistically significantly lower than the control ($p \leq 0.05$) with a 23 % decrease. None of the other species were adversely affected and a 21 % increase in soybean occurred.

Conclusions:

No detrimental effect greater than 23 % was observed in any of the parameters monitored for any of the tested species following an exposure to Condor OF (non-aqueous flowable concentrate formulation containing *Bacillus thuringiensis* kurstaki EG2348). Two species, cabbage and corn (maize), were statistical significantly affected, to a minor extent, ,as compared to control plants. The emergence of seedlings was the most sensitive parameter recorded.

Table 9.7-1 Percentage effect compared to control on seedling germination and emergence parameters following a treatment with Condor OF (non-aqueous flowable concentrate formulation containing *Bacillus thuringiensis* as a.s.) at a rate equivalent to 348 g a.s./ha or a blank control

Plant species	Radicle length	Germination	Phytotoxicity rating	Emerged seedlings	Seedling height	Dry weight
Soybean (*Glycine max*)	11	4	0.1	-8	6	21
Lettuce (*Lactuca sativa*)	9	-2	0.0	-18	10	20
Carrot (*Daucus carota*)	6	8	0.0	-7	0	0
Tomato (*Lycopersicon esculentum*)	12	4	0.0	4	8	0
Cucumber (*Cucumis sativus*)	3	2	0.1	0	2	6
Cabbage (*Brassica oleracea*)	8	-9	0.3 *	-2	2	-23 *
Oat (*Avena sativa*)	-6	2	0.0	0	-1	1

Perennial Ryegrass (*Lolium perenne*)	5	2	0.1	-2	-3	0
Corn (maize) (*Zea mays*)	-1	0	0.3	-16 *	3	13
Onion (*Allium cepa*)	-2	-8	0.1	-2	4	0

Values represent results from the means of five replicates per treatment group (ten seeds per replicate) at the end of respective incubation periods

* statistically significantly different to the control, Duncan's Multiple Range Test ($p \leq 0.05$)

KMA 9.7/02: Nontarget phytotoxicity test: Vegetative vigour, Tier 2. (Cañez, V.M., 1988b)

Guidelines: FIFRA 122-1

GLP: Yes

Materials and methods:

Test substance: B. thuringiensis kurstaki EG2348 formulated as Condor OF – a non-aqueous flowable concentrate.

Content of a.i.: Not stated

Test species: Six dicotyledon species and four monocotyledon plant species: Soybean (*Glycine max*), Lettuce (*Lactuca sativa*), Carrot(*Daucus carota*), Tomato (*Lycopersicon esculentum*), Cucumber (*Cucumis sativus*), Cabbage(*Brassica oleracea*), Oat (*Avena sativa*), Perennial Ryegrass (*Lolium perenne*), Corn (maize)(*Zea mays*) and Onion (*Allium cepa*).

Number of organisms, size: Pots containing five seedling plants each per pot at the 1 to 3 true leaf stage. Three replicate pots were used per species per concentration

Applied and measured concentrations: Not specifically stated. Condor OF was applied at application rates corresponding to 44, 87, 175, 348 and 697 g a.s./ha onto pots.

Test conditions. During the 21-day incubation, the plastic test pots were kept in a greenhouse under natural light conditions which averaged 12.8 hours light per day at a mean intensity of 15,600 to 22,200 lux and watered from the top via regular irrigation which was adjusted as necessary. The test soil was a general purpose potting soil containing fir bark, redwood, Canadian sphagnum peat moss and sand.

Findings:

Mean phytotoxicity ratings were 0.0 for all plant species apart for corn (maize) which had a rating of 0.3. Similarly, plant height increase from day 0 to 21 was not statistically significantly affected in any species or at any treatment rate. Dry weight in lettuce was statistically significantly lower than the control at treatment rates of 175 and 697 g a.s./ha with a decrease of 28 and 23 %, respectively. None of the other species were statistically significantly affected compared to the control. The dry weight of onion plants treated with 44 and 88 g a.s./ha was statistically significantly lower than onion plants treated with 348 and 697 g a.s./ha

Conclusions:

No effect on plant seedlings with various rates of Condor OF (non-aqueous flowable concentrate formulation containing *Bacillus thuringiensis kurstaki* EG2348), was seen in terms of plant height. Therefore, effects on plant height lie above the highest tested rate of 697 g a.s./ha for all species. For plant dry weight for all species tested apart from lettuce an effect lie above the highest tested rate of 697 g a.s./ha. The effect on lettuce was determined to be 348 g a.s./ha.

New information

No further new studies were provided. For first approval of Btk strains SA-11, SA-12 and EG2348 effects on terrestrial plants were evaluated. *Bacillus thuringiensis* subsp. *kurstaki*, strain SA-12 is toxic specifically to insects of the *Lepidopteran* order and no effects on aquatic plants from applications of Btk in insecticidal formulations targeted specifically at these insects is expected or envisaged. Furthermore, the lack of reports of negative effects on plants from numerous studies on the persistence and fate of Btk on plants, as well as lack of reported negative effects from decades of use in agricultural and forestry environments indicates that no adverse effect

occurs on plants. Two studies on the effects of a product based on Btk EG2348 on seedling emergence and vegetative vigour were evaluated in the DAR (2008). No considerable adverse effects on plant seedlings and vegetative vigour were observed.

B.9.8 References relied on

Several literature review reports have been provided according to the guidance of EFSA (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). The aim of these reports was to provide a global overview of peer-reviewed literature concerning potential side effects of *B. thuringiensis* subsp. *kurstaki* strain SA-12.

Overview of literature reports provided according to the guidance of EFSA

Data point	Author	Year	Title	Section of RMS evaluation
KMA 2.7/12 & 3.5/06	Süß, J.	2016	Literature review on *B. thuringiensis* subsp. *kurstaki* strain SA-12: Biological properties	Vol. 3MA, B.2.10
KMA 6.1.1/06	Seehase, S.	2016	Literature review on *B. thuringiensis* subsp. *kurstaki* strain SA-12: Toxicology	Vol. 3MA, B.6.3
KMA 7.1/01	Cornelese, A.	2016a	Literature review on *B. thuringiensis* subsp. *kurstaki* strain SA-12 and metabolites: Residues in or on treated products, food and feed	Vol. 3MA, B.7.4
KMA 8.1/10	Cornelese, A.	2016b	Literature review on *B. thuringiensis* subsp. *kurstaki* strain SA-12: Fate and behaviour in the environment	Vol. 3MA, B.8.3
KMA 9/01	Schöbinger, U.	2016	Literature review on *B. thuringiensis* subsp. *kurstaki* strain SA-12: Effects on non-target organisms	Vol. 3MA, B.9.8

Literature review on *B. thuringiensis* subsp. *kurstaki* strain SA-12: "Effects on non-target organisms" (Schöbinger, U, 2016)

RMS comments on the literature search: "Literature review on *B. thuringiensis* subsp. *kurstaki* strain SA-12: Effects on non-target organisms". (Schöbinger, U., 2016; submitted in Data point KMA 8.01	The review was made in order to identify scientific peer-reviewed open literature on the active substance *Bacillus thuringiensis* subsp. *kurstaki* SA-12 which may affect the assessment on non-target organism. The search strategy was based on a multi-concept approach. **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 organisms not known as beneficial organism or desribed as pest • Non-target organisms 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. *kurstaki* pathogens in a certain non-target organisms is there any relevance for *Bacillus thuringiensis* subsp. *kurstaki* **Database searched** A search was conducted using the DIMDI database provided by the German Institute of Medical Documentation and comprised of searches in MEDLINE, BIOSIS, CAB and SCISEARCH databases **Search methods**

	Search strategy aimed to find all recent (from 2006 onwards) references that are of ecotoxicological relevance, regarding possible effects on non-target organisms. The following keywords were used in the searches: *Bacillus thuringiensis kurstaki*, bird? Aves?, fish? Daphnia, alga?, aquatic plant?, phhytotox?, phytopathogen?, bee, bees honeybees, bu,blebees, arthropod?, insect?, tox, pathogen?, NOT Bt-maize, 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. **Results of the study selection process** In total 553 references were retrieved and 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. In total 14 references were identified as being potentially relevant. These references were subjected to a full-text assessment in Step 2. Ten references were finally classified as relevant and supportive and are included in the dossier.
Conclusion	The literature search, regarding data on possible effects on non-target organisms, was accepted as valid, both regarding inclusion of databases and use of search terms. By the searches for “Effects on non-target organisms” 10 references were finally considered relevant and reliable and are included in the dossier.

Reference list

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	Previous evaluation
KMA 9/01	Schöbinger, U.	2016	LITERATURE REVIEW ON BACILLUS THURINGIENSIS SUBSP. KURSTAKI STRAIN SA-12: EFFECTS ON NON-TARGET ORGANISMS Certis USA LLC GAB Consulting GmbH, Stade, Germany Report-no.: 228-1384-MA-08-01_SA-12 GLP/GEP: no Published: no	no	yes	protected	Certis USA	New data for active ingredient, not previously submitted nor evaluated
KMA 9.1/01	Buckner, C.H., Kingsbury, P.D., Mcleod, B.B., Mortensen, K.L., Ray, D.G.H	1974	EVALUATION OF COMMERCIAL PREPARATIONS OF BACILLUS THURINGIENSIS WITH AND WITHOUT CHITINASE AGAINST SPRUCE BUDWORM. F. IMPACT OF AERIAL TREATMENT ON NON-TARGET ORGANISMS, ALGONQUIN PARK, ONTARIO AND SPRUCE WOODS, MANITOBA Chemical Control Research Institute, Canadian Forestry Service, Ottawa, Ontario, Canada, 1974 Report-no.: Information Report CC-X-59 GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 9.1/02	Nagy, L.R., Smith, K.G.	1997	EFFECTS OF INSECTICIDE-INDUCED REDUCTION IN LEPIDOPTERAN LARVAE ON REPRODUCTIVE SUCCESS OF HOODED WARBLERS The Auk,1997, Volume 114, pp. 619-627 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 9.1/03	[redacted]	2015 a	THURICIDE SC - AVIAN (COTURNIX COTURNIX JAPONICA) ORAL ACUTE TOXICITY/PATHOGENICITY STUDY Certis USA LLC, RL1364/2015PAVO-B [redacted] GLP: yes Published: no	yes	yes	protected	Certis USA	New data for active ingredient, not previously submitted nor evaluated

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	Previous evaluation
KMA 9.2.1/01	[redacted]	2015 b	THURICIDE SC - FISH (DANIO RERIO) TOXICITY TEST Certis USA LLC, RL1371/2015PX-B [redacted] GLP: yes Published: no	yes	yes	protected	Certis USA	New data for active ingredient, not previously submitted nor evaluated
KMA 9.2.2/01	Eidt, D.C.	1985	TOXICITY OF BACILLUS THURINGIENSIS VAR. KURSTAKI TO AQUATIC INSECTS The Canadian Entomologist, Volume 117, pp. 829-837 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 9.2.2/02	Melin, B.E., Cozzi, E.M.	1990	SAFETY TO NONTARGET INVER-TEBRATES OF LEPIDOPTERAN STRAINS OF BACILLUS THURINGIENSIS AND THEIR BETA-EXOTOXINS Safety of Microbial Insecticides (M. Laird, *et al*., editors), CRC Press, Boca Raton, Florida, USA, pp. 149-167 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 9.2.2/03	Kreutzweiser, D.P., Capell, S.S., Thomas, D.R.	1994	AQUATIC INSECT RESPONSE TO BACILLUS THURINGIENSIS VAR. KURSTAKI IN A FOREST STREAM Canadian Journal of Forest Research, Volume 24, pp. 2041-2049 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 9.2.2/04	Kreutzweiser, D.P., Holmes, S.B., Capell, S.S., Eichenberg, D.C.	1992	LETHAL AND SUBLETHAL EFFECTS OF BACILLUS THURINGIENSIS VAR KURSTAKI ON AQUATIC INSECTS IN LABORATORY BIOASSAYS AND OUTDOOR STREAM CHANNELS Bulletin of Environmental Contamina-tion and Toxicology, Volume 49, pp. 252-258 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008

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	Previous evaluation
KMA 9.2.2/05	Mrotzeck Masquetto, A.	2015	THURICIDE SC - DAPHNIA MAGNA TOXICITY TEST Certis USA LLC, RL1339/2015DP-B TECAM Tecnologia Ambiental, Sao Paulo, Brasil GLP: yes Published: no	no	yes	protected	Certis USA	New data for active ingredient, not previously submitted nor evaluated
KMA 9.2.2/06	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 Ecotoxicology, 20, 1354-1360 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated
KMA 9.2.3/01	Koskella, J., Stotzky, G.	2002	LARVICIDAL TOXINS FROM BACILLUS THURINGIENSIS SUBSPP. KURSTAKI, MORRISONI (STRAIN TENEBRIONIS), AND ISRAELENSIS HAVE NO MICROBICIDAL OR MICROBIOSTATIC ACTICITY AGAINST SELECTED BACTERIA, FUNGI AND ALGAE IN VITRO Canadian Journal of Microbiology, Volume 48, pp. 262-267 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 9.3/01	Buckner, C.H., Kingsbury, P.D., Mcleod, B.B., Mortensen, K.L., Ray, D.G.H.	1974	EVALUATION OF COMMERCIAL PREPARATIONS OF BACILLUS THURINGIENSIS WITH AND WITHOUT CHITINASE AGAINST SPRUCE BUDWORM. F. IMPACT OF AERIAL TREATMENT ON NON-TARGET ORGANISMS, ALGONQUIN PARK ONTARIO AND SPRUCE WOODS, MANITOBA Chemical Control Research Institute, Canadian Forestry Service, Ottawa, Ontario, Canada, 1974 Report-no.: Information Report CC-X-59 GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008

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	Previous evaluation
KMA 9.3/02	Malone, L.A., Burgess, E.P.J., Stefanovic, D.	1999	EFFECTS OF A BACILLUS THURINGINSIS TOXIN, TWO BACILLUS THURINGIENSIS BIOPESTICIDE FORMULATIONS, AND A SOYBEAN TRYPSIN INHIBITOR ON HONEY BEE (APIS MELLIFERA L.) SURVIVAL AND FOOD CONSUMPRION Apidologie, Volume 30, pp. 465-473 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 9.3/03	Cantwell, G.E., Lehnert, T., Fowler, J.	1972	ARE BIOLOGICAL INSECTICIDES HARMFUL TO THE HONEY BEE? American Bee Journal, pp. 255-258 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 9.3/04	Lehnert, T., Cantwell, G.E.	1978	THE EFFECTS OF MICROBIAL INSECTICIDES ON THE HONEY BEE - A REVIEW American Bee Journal, October 1978, pp. 674-675 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 9.3/05	Krieg, A.	1973	ÜBER DIE TOXISCHE WIRKUNG VON BACILLUS CEREUS – UND BACILLUS THURINGIENSIS-KULTUREN AUF DIE HONIGBIENE (APIS MELLIFERA) Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz, Volume 8, pp. 483-486 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 9.3/06	Minei, C.C.	2015 a	THURICIDE SC - HONEYBEES (APIS MELLIFERA), ACUTE ORAL TOXICITY TEST Certis USA LLC, RL1299/2015ABO-B TECAM Tecnologia Ambiental, Sao Paulo, Brasil GLP: yes Published: no	no	yes	protected	Certis USA	New data for active ingredient, not previously submitted nor evaluated
KMA 9.3/07	Minei, C.C.	2015 b	THURICIDE SC - HONEYBEES (APIS MELLIFERA), ACUTE CONTACT TOXICITY TEST Certis USA LLC, RL1300/2015ABC-B TECAM Tecnologia Ambiental, Sao Paulo, Brasil GLP: yes Published: no	no	yes	protected	Certis USA	New data for active ingredient, not previously submitted nor evaluated

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	Previous evaluation
KMA 9.3/08	Mayer, D.F.	1990	EFFECT OF SANDOZ BT PRODUCTS ON ADULT HONEY BEE (APIS MELLIFERA L.) MORTALITY Certis USA LLC, 90/01 GLP: yes Published: no	no	yes	protected	Certis USA	New data for active ingredient, not previously submitted nor evaluated
KMA 9.3/09	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) Spanish J. of Agricultural Research, 12(2), 405-408 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated
KMA 9.3/10	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 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated
KMA 9.4/01	Melin, B.E., Cozzi, E.M.	1990	SAFETY TO NONTARGET INVER-TEBRATES OF LEPIDOPTERAN STRAINS OF BACILLUS THURINGIENSIS AND THEIR BETA-EXOTOXINS Safety of Microbial Insecticides (M. Laird, *et al*., editors), CRC Press, Boca Raton, Florida, USA, pp. 149-167 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008

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	Previous evaluation
KMA 9.4/02	Buckner, C.H., Kingsbury, P.D., Mcleod, B.B., Mortensen, K.L., Ray, D.G.H.	1974	EVALUATION OF COMMERCIAL PREPARATIONS OF BACILLUS THURINGIENSIS WITH AND WITHOUT CHITINASE AGAINST SPRUCE BUDWORM. F. IMPACT OF AERIAL TREATMENT ON NON-TARGET ORGANISMS, ALGONQUIN PARK ONTARIO AND SPRUCE WOODS, MANITOBA Chemical Control Research Institute, Canadian Forestry Service, Ottawa, Ontario, Canada, 1974 Report-no.: Information Report CC-X-59 GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 9.4/03	Hamed, A.R.	1979	ZUR WIRKUNG VON BACILLUS THURINGIENSIS AUF PARASITEN UND PRÄDATOREN VON YPONOMEUTA EVONYMELLUS (LEP. YPONOMEUTIDAE) Zeitschrift für Angewandte Entomologie, 1978/1979, Volume 87, pp. 294-311 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 9.4/04	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) Journal of Economic Entomology, 103(6), 2061-2071 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated
KMA 9.4/05	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, 29(4), 355-359 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated

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	Previous evaluation
KMA 9.4/06	Garcia, P.V., Pereita, N., Oliveira, L.M.	2008	SIDE-EFFECTS OF ORGANIC AND SYNTHETIC PESTICIDES ON COLD-STORED DIAPAUSING PREPUPAE OF TRICHOGRAMMA CORDUBENSIS BioControl, 54, 451-458 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated
KMA 9.4/07	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, 26 (5), 678-690 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated
KMA 9.4/08	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 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated
KMA 9.4/09	Amichot, M., Curty, 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 & Pollution Research 23(4), 3097-3103 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated
KMA 9.5/01	Bilej, M., Procházková, P., Šilerová, M., Josková, R.	2010	EARTHWORM IMMUNITY Invertebrate Immunity, Kenneth Söderhäll (ed.), Landes Bioscience and Springer Science+Business Media, 66-79 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated

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	Previous evaluation
KMA 9.6/01	O'Callaghan, M., Gerard, E., Sarathchandra, U.	2007	ANALYSIS OF NON-TARGET IMPACTS OF FORAY 48B ON SOIL MICRO-ORGANISMS Proceedings of the 6th PRC on the Biot. of *B. thur.* and its Env. Impact, 133-134 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated
KMA 9.6/02	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 Biocontrol Science and Technology, 21, 1225-1242 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated
KMA 9.7/01	Cañez, V.M.	1988 a	NONTARGET PHYTOTOXICITY TEST: VEGETATIVE VIGOUR, TIER 2 Pan-Agricultural laboratories, Inc., Fresno, California, USA Certis USA LLC, Columbia Report-no.: LR88-52 GLP/GEP: yes Published: no	no	no	not protected	Certis USA	DAR 2008
KMA 9.7/02	Cañez, V.M.	1988 b	NONTARGET PHYTOTOXICITY TEST: SEED GERMINATION/SEEDLING EMERGENGE, TIER 1 Pan-Agricultural laboratories, Inc., Fresno, California, USA Certis USA LLC, Columbia Report-no.: LR88-53 GLP/GEP: yes Published: no	no	no	not protected	Certis USA	DAR 2008