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.3 (MPCA)

Data on application

Rapporteur Member State: Denmark

Co- Rapporteur Member State: The Netherlands

Version history

When	What
2008	DAR
2019	Initial RAR

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B.3 Data on application

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 Subsequently Regulation (EC) No 1107/2009 repealed and replaced Directive 91/414/EEC and the active substance Btk SA-12, was deemed to be approved under that Regulation and included in the Annex to Regulation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.

As the manufacturing process of Btk SA-12 has not been changed since original approval, all data submitted for the original approval of the strain are considered fully applicable for the current evaluation.

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.

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

B.3.1 Function

Btk SA-12 is used for control of insects of the order Lepidoptera.

B.3.2 Field of use envisaged

Btk SA-12 is intended for use as an insecticide in agriculture, horticulture and viticulture.

B.3.3 Crops or products protected or treated

Btk SA-12 is used as an insecticide in a wide range of crops. With this dossier the proposed GAP of *Bacillus thuringiensis* subsp. *kurstaki* SA-12 includes pome fruits, protected tomato and ornamentals.

B.3.4 Method of production and quality control

CONFIDENTIAL information. Please refer to Vol. 4, C.1.3.2.

B.3.5 Information on the occurrence or possible occurrence of the development of resistance of the target organism(s)

Due to the well-known history of resistance development in the field of traditional chemical insecticides, the question of insect resistance development to Bt arises. However, Bt is very unlike other insecticides in its origin and mode of action. Insect pests developing resistance to other insecticides is, in fact, one of the many reasons *Bacillus thuringiensis* has come into common use today

The RMS evaluated the risk of resistance development of *B. thuringiensis* subsp. *kurstaki* SA-11, SA-12 and EG2348 as minimal in the original DAR 2008.

After over 40 years of wide-spread use on a global basis, development of resistance to *B. thuringiensis* subsp. *kurstaki* in the field has been very limited. Commercial formulations of *B. thuringiensis* had been used for more than 20 years before the first cases of substantial resistance to *B. thuringiensis* were documented in open field populations of any pest.

In the mid-1980s a number of insect populations of several different species with different levels of resistance to *B. thuringiensis* crystal proteins were obtained by laboratory selection experiments: the Indian-meal moth (*Plodia interpunctella*), tobacco budworm (*Heliothis virescens*), beet armyworm (Spodoptera exigua), cabbage looper (*Trichoplusia ni*) and the diamondback moth (*Plutella xylostella*) (reviewed in Schnepf et al., 1998). However, only two insect species have been reported to have developed resistance under “field” conditions: the diamondback moth, *Plutella xylostella*, in several countries (Tabashnik, 1994), and the cabbage looper, *Trichoplusia ni*, in greenhouse vegetable production in Canada (Janmaat & Myers, 2003).

Mechanisms of resistance

Studies on resistance mechanisms most commonly have shown the resistance mechanisms to involve a change in the membrane receptors to which activated Bt toxin binds. E.g. resistance to *B. thuringiensis* in *P. xylostella* is associated with reduced binding of ICP to the brush border membranes of midgut epithelium (Ferré et al., 1991).

The complex mode of action of *B. thuringiensis*, often involving multiple toxins and *B. thuringiensis* spores, is supposed to provide protection against resistance because a single mutation in the insect would be unlikely to affect susceptibility. McGaughey & Whalon (1992) however, suggested that at high levels of selection, the multicomponent-toxicity pathway merely expands behavioural and /or physiological opportunities for adaptation to *Bt*.

The field evolved resistance to *B. thuringiensis* toxins depends on the crystal toxin, the combination of crystal toxins and the target organism.

One of the simplest resistance management strategies may be more useful for extending the effectiveness of *Bt* than previously thought, is temporal refugia from exposure to *B. thuringiensis*. Tabashnik et al. (1994) found that the resistance evolution can be reversible. A rapid reversal up to 2800-fold resistance to *B. thuringiensis* in *P. xylostella* in the absence of exposure to ICPs was associated with restoration of ICP binding and increased biotic fitness. A rapid decline of resistance in populations of cabbage loopers, *Trichoplusia ni* collected from greenhouses and maintained in the laboratory without selection could be reported by Janmaat et al (2003). Management of pests resistant to *Bt* in vegetable greenhouses will require sporadic use of *Bt*-based sprays or alternatively use of sprays containing other *Bt* toxins.

Thus, the development of resistance in target pests is possible with *B. thuringiensis* subsp. *kurstaki* and standard resistance risk management strategies such as altering of control strategies with different modes of action, reduction of selection pressure, use of refugia for susceptible biotypes and monitoring methods should be observed. Due to the different mode of action of *B. thuringiensis* subsp. *kurstaki* compared to conventional insecticides, the risk of pest populations developing cross resistance is very low. Thus, *B. thuringiensis* subsp. *kurstaki* can be considered a valuable part in resistance management strategies.

New information

A literature search according to EFSA (2011)[2] guidance was carried out to obtain any new information relevant for the data point. The search was conducted in May 2016 using the DIMDI database provided by the German Institute of Medical Documentation and comprised searches in MEDLINE, BIOSIS, CAB and SCISEARCH databases. The search was done at subspecies level and included typical terms targeting references on resistance development in target insects. From a total of 241 hits (after removal of doubled) 16 were submitted to full text analysis and 12 were finally considered relevant and reliable and are included in the dossier. During the assessment, all references referring to resistance mechanisms and Bt-transgenic crops were excluded. In addition, laboratory studies selecting for resistance were excluded. For more details, please refer to the literature review report by Süß (2016, KMA 3.5/06).

Previously, development of resistance under field conditions was reported for *Plutella xylostella* and *Trichoplusia ni* in various countries. Besides these two species, the literature research revealed one record on resistant field-populations of *Helicoverpa armigera* in India. It is noteworthy, that not a single report on resistant insect populations in Europe was obtained. The reported cases of resistant field populations are summarised in **Table 3.5-1** below. The references also demonstrate that resistance to single Cry proteins is more commonly found than resistance to Btk (e.g. Wang et al., 2007; Mittal et al., 2007; Gong et al. 2010) confirming the complexity of the insecticidal activity of Btk. A study of Janmaat et al. (2015) demonstrates that alternating use of Bt products with other biological or chemical insecticides can reduce resistance in *T. ni* populations considerably.

Table 3.5-1 Overview of reports on resistant insect populations obtained by a literature search according to EFSA guidance

Pest	Country	Degree of resistance	Remarks	Reference
Plutella xylostella	China	Decreased susceptibility - moderate resistance (RR = 3.8 - 35.3)	Test with commercial product	Jiang et al. (2015)
Plutella xylostella	China	RR 69.92 - 252.35	Population monitoring in four regions over three years, high levels of resistance at all samplings.	Xia et al. (2014)
Plutella xylostella	China	RR 1.00 - 21.92	Commercial Btk-based formulation was used, resistance to single cry toxins was much higher (RR Cry1Ac: 2.45 -56.15; RR Cry1Ba 1.18 - 6.17).	Wang et. al (2007)
Plutella xylostella	China	RR 6	Resistance to single Cry toxins was much higher (e.g. Cry1Ab = 100 and Cry1Ac = 30), the field population was used for selection of a resistant strain for further laboratory experiments.	Gong et al. (2010)

[2] 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

Plutella xylostella	India	RR 1.0 - 7.82	Reduced susceptibility correlated with temperature with higher susceptibility at lower temperatures, insects were more resistant to single purified cry toxins than to the spore-crystal complex.	Mittal et al., (2007)
Plutella xylostella	Philippines	RR 1.7 - 5.3	Commercial Bt product was used.	Sarmiento & Ocampo (2010)
Plutella xylostella	India	RR 0.93 - 47.03	Spatial differences in resistance with highest RR found for regions with extensive Bt use, commercial products (Biobit, Dipel 8L, Delfin) were used.	Pereira et al. (2006)
Plutella xylostella	India	Btk RR 8 - 15 Bta RR 1.9	Commercial products used: Biobit, Dipel, Centari	Sannaveerappanavar & Virktamath, (2006)
Plutella xylostella	Brazil	RR Btk 1.0 - 5.8 RR Bta 1.0 - 54.0	RR based on LC_{50} values; commercial products were tested (Dipel, Xentari), higher resistance for Bta caused by heavy use of Bta-based ppp in some areas; preference of untreated areas for egg laying, reduced oviposition in treated areas.	Zago et al., (2014)
Trichoplusia ni	USA/ Canada	RR not provided, high resistance in single greenhouse colonies	Resistance in greenhouse populations higher than in field populations, correlation with genetic diversity, highest resistance in overwintering greenhouse populations.	Franklin et al. (2010)
Trichoplusia ni	Canada	RR not provided, decrease in resistance by time	Higher resistance in greenhouses, decrease by time due to use of other insecticides than Bt, no spread of resistance from greenhouse to field populations.	Janmaat et al. (2015)
Helicoverpa armigera	India	RR 5.7 - 119.5	Insect populations were collected from areas where Bt cotton is grown. Additionally, the susceptibility to Bt/Cry1Ac as well as to Cry1Ac alone and Bt-cotton leaves were assessed.	Kalia et al. (2006)

RR: resistance ratio, LC_{50} of field strain/LC_{50} of susceptible strain (RR scoring according to Guideline for Resistance Monitoring of DBM in Cruciferous Vegetables (RR scoring < 3 = susceptible, 3.1-5.0 = decreased susceptibility, 5.1 - 10 = low resistance, 10.1 - 40 = moderate resistance, 40.1 - 160 = high resistance, > 160 very high resistance)

RMS evaluation	We have no remarks to the information and references referred to in the original DAR of Btk strains SA-11, SA-12 and EG2348. We find the information relevant and still valid for renewal of Btk SA-12.
RMS conclusion	In Europe no reports on resistant insect populations were obtained. Btk SA-12 is a microbial disruptor of insect midgut membranes Due to the different mode of action of *B. thuringiensis* subsp. *kurstaki* compared to conventional insecticides, the risk of pest populations developing cross resistance is very low. Thus, *B. thuringiensis* subsp. *kurstaki* can be considered a valuable part in resistance management strategies. However, Btk products like any other insecticide should be used in IRM (Insecticide Resistance Management) or IPM (Integrated Pest Management) programs and not used over and over as the only insecticide of choice.

Cited literature abstracts:

Report:	KMA 3.5/06 - Süß, J. (2016)
Title:	Literature review on *Bacillus thuringiensis* subsp. *kurstaki* SA-12 Biological properties
Document No:	2281384-MA-02-01_SA-12
Abstract	Not available

Evaluation RMS	Please see Vol. 3MA, B.2.10 for evaluation of the literature review.

Report:	KMA 3.5/11 - Jiang, T., Wu., S., Yang, T., Zhu, C., Gao, C. (2015) Florida Entomologist, 98(1):65-73, Published report
Title:	Monitoring field populations of *Plutella xylostella* (Lepidoptera: Plutellidae) for resistance to eight insecticides in China
Abstract	The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is the main destructive insect pest of brassicaceous vegetables around the world. It has developed resistance to various classes of insecticides. However, the current status of insecticide resistance in *P. xylostella* has not been examined in China. In this study, concentration-mortality responses of *P. xylostella* to 8 insecticides, including abamectin, chlorantraniliprole, spinosad, beta-cypermethrin, chlorfenapyr, diafenthiuron, chlorfluazuron and the bio-pesticide *Bacillus thuringiensis kurstaki* (Btk) were evaluated. The results showed that almost all of the tested populations had developed high to very high resistance to abamectin and beta-cypermethrin, with resistance ratios ranging from 62.9 to 1494.7-fold. Chlorantraniliprole was very effective against *P. xylostella* in most tested populations except those from Taihe and Wuxi. Approximately 61% of tested populations displayed moderate resistance to spinosad, while other field populations showed minor changes (3-fold) in their susceptibility to this insecticide. Obvious variation (93-fold) of susceptibility to chlorfenapyr existed in field populations of which 32% displayed low level resistance, and 36% exhibited moderate resistance. Only one field population (Wuxi) showed very high resistance to chlorfenapyr (RR = 260.1). Diafenthiuron and chlorfluazuron were highly effective against all of the tested populations with resistance ratios (RR) ranging from 0.4 to 8.7 – fold. Decreased susceptibility ranging to moderate resistance to Btk was observed (RR = 3.8 – 35.3). Significant correlations were detected between the values of logLC_{50} of chlorantraniliprole and 4 insecticides (abamectin, spinosad, beta-cypermethrin and chlorfenapyr). The results of this study provided valuable information for choosing alternative insecticides and for integrated resistance management of *P. xylostella*.

Evaluation RMS	No remarks

Report:	KMA 3.5/12 - Xia, Y., Lu, Y., Shen, J., Gao, X., Qiu, H., Li, J. (2014) Crop Protection 63:131-137, Published report
Title:	Resistance Monitoring for eight insecticides in *Plutella xylostella* in central China
Abstract	The diamondback moth, *Plutella xylostella* L., is one of the most important pests of cruciferous vegetables in the world. Assessment of changing insecticide resistance is essential for maintaining control efficiency and resistance management. In this study, four field populations of *P. xylostella* were collected from cabbage (*Brassica oleracea*, var. *capitata* L.) fields in central China from 2010 to 2012 to monitor their resistance to abamectin, *Bacillus thuringiensis* (BT) subsp. *kurstaki* (WG-001), spinosad, chlorfluazuron, chlorfenapyr, diafenthiuron, indoxacarb and beta-cypermethrin by using a leaf-dipping bioassay method. The results indicated that chlorfenapyr and diafenthiuron showed high toxicity to *P. xylostella* in central China, with no obvious toxicity change during the three years. The resistance of *P. xylostella* to spinosad was at low to moderate levels of resistance in all three years. Resistance of *P. xylostella* to abamectin, chlorfluazuron and indoxacarb varied greatly among the four regions. *P. xylostella* exhibited moderate and high levels of resistance to abamectin, chlorfluazuron and indoxacarb. The resistance of this pest to BT (WG-001) and beta-cypermethrin was severe in the four regions. Our study was conducive for developing a more effective resistance management program for *P. xylostella*.

Evaluation RMS	No remarks

Report:	KMA 3.5/07 - Wang, L., Li, X.-F., Zhang, J., Zhao, J.-Z., Wu, Q.-J., Xu, B., Zhang, Y.-J. (2007) Journal of Applied Entomology 131(7):441–446, Published report
Title:	Monitoring of resistance for the diamondback moth to *Bacillus thuringiensis* Cry1Ac and Cry1Ba Toxins and a Bt Commercial formulation
Abstract	To monitor the resistance of field populations of the diamondback moth *Plutella xylostella* in China to the insecticidal protein Cry1Ac, Cry1Ba and commercial formulation *Bacillus thuringiensis* var. *kurstaki* (Btk), six representative populations of the diamondback moth were collected from Shanghai, Shandong, Hubei, Hunan, Zhejiang and Guangdong provinces of China where crucifer crop plants are intensively planted. Bioassay results showed that the populations of the diamondback moth from different locations exhibited different levels of resistance, compared with a susceptible laboratory population. The Guangdong field population was 56.15- and 21.90-fold resistant to Cry1Ac and Btk, respectively. Shanghai, Hunan, Shandong and Zhejiang populations were 37.85-, 17.24-, 10.24- and 9.41-fold resistant to Cry1Ac, respectively, but were not resistant to Btk. The Hubei population did not show resistance to Cry1Ac and Btk. Almost all tested populations were susceptible to Cry1Ba, but the Guangdong population showed some tolerance to Cry1Ba with a LC_{50} of 0.69 μg/mL which was 6.17-fold higher than that of the susceptible population. The results suggested that the complex resistance patterns of field populations of *P. xylostella* need to be considered for expression of Bt toxin genes in genetically-engineered crop plants and commercial formulations.

Evaluation RMS	No remarks

Report:	KMA 3.5/09 - Gong, Y., Wang, C., Yang, Y., Wu, S., Wu, Y. (2010) Journal of Invertebrate Pathology, 104(2):90-96., Published report
Title:	Characterization of resistance to *Bacillus thuringiensis* toxin Cry1Ac in *Plutella xylostella* from China
Abstract	A field population (SZ) of *Plutella xylostella*, collected from the cabbage field in Shenzhen, Guangdong Province of China in 2002, showed 2.3-fold resistance to Cry1Aa, 110-fold to Cry1Ab, 30-fold to Cry1Ac, 2.1-fold to Cry1F, 5.3-fold to Cry2Aa and 6-fold resistance to *Bacillus thuringiensis* var. *kurstaki* (Btk) compared with a susceptible strain (ROTH). The SZBT strain was derived from the SZ population through 20 generations of selection with activated Cry1Ac in the laboratory. While the SZBT strain developed 1200-fold resistance to Cry1Ac after selection, resistance to Cry1Aa, Cry1Ab, Cry1F, and Btk increased to 31-, 1900-, > 33- and 17-fold compared with the ROTH strain. However, little or no cross-resistance was detected to Cry1B, Cry1C and Cry2Aa in the SZBT strain. Genetic cross analyses between the SZBT and ROTH strains revealed that Cry1Ac-resistance in the SZBT strain was controlled by a single, autosomal, incompletely recessive gene. Binding studies with (125)I-labeled Cry1Ac showed that the brush border membrane vesicles (BBMVs) of midguts from the resistant SZBT insects had lost binding to Cry1Ac. Allelic complementation tests demonstrated that the major Bt resistance locus in the SZBT strain was same as that in the Cry1Ac-R strain which has "mode 1" resistance to Bt. An F(1) screen of 120 single-pair families between the SZBT strain and three field populations collected in 2008 was carried out. Based on this approach, the estimated frequencies of Cry1Ac-resistance alleles were 0.156 in the Yuxi population from Yunnan province, and 0.375 and 0.472 respectively in the Guangzhou and Huizhou populations from Guangdong province.

Evaluation RMS	No remarks

Report:	KMA 3.5/08 - Mittal, A., Kumari, A., Kalia, V., Kumar Singh, D., Gujar, G.T. (2007) Biopesticides International, 3(1):58-70, Published report
Title:	Spatial and temporal baseline susceptibility of diamondback moth, *Plutella xylostella* (Linnaeus) to *Bacillus thuringiensis* spore Crystal mixture, purified Crystal Toxins and mixtures of cry Toxins in India
Abstract	Toxicity of purified crystal proteins and spore crystal preparations of *Bacillus thuringiensis* subsp. *kurstaki* was evaluated against six-day-old larvae of the diamondback moth, *Plutella xylostella* (Linnaeus) collected from fifteen different locations in India, using the leaf dip bioassay method. The LC_{50} (ng/mL, 72 h) values of *B. thuringiensis* Cry proteins for different populations of *P. xylostella* ranged from 3.6 – 28.0 ng/mL for HD-1, 5.8 – 485.5 ng/mL for Cry1Ab and 12.2 – 86.4 ng/mL for Cry1C. *B. thuringiensis* subsp. *kurstaki* HD-1 spore crystal mixture was more toxic than Cry1 toxins, viz. Cry1Ab and Cry1C. The mean LC_{99} of HD-1 spore crystal complex, Cry1Ab and Cry1C were 344.1, 1257.4 and 9323.6 ng/mL, respectively, which could be used as discriminating doses for routine resistance monitoring of *P. xylostella*. Temporal variation in susceptibility of *P. xylostella* collected from Najafgarh fields showed a positive temperature relationship with *B. thuringiensis* toxicity. Susceptibility of larvae was more apparent during the early season (onset of winter), to both *B. thuringiensis* subsp. *kurstaki* HD-1 and Cry1Ab, *versus* late season or late winter. The LC_{50} for Cry1C was less during late winter. Mixtures of Cry1C with Cry1Ab or Cry9A were synergistic, whereas the Cry1Ab and Cry9A mixture was additive in toxicity to *P. xylostella* larvae, compared to their individual component toxins. These studies are discussed in relation to the continued reliance on sprayable *B. thuringiensis* subsp. *kurstaki* formulations, and future cultivation of *B. thuringiensis* transgenic cole crops in the country.

Evaluation RMS	No remarks

Report:	KMA 3.5/13 - Sarmiento, G.M., Ocampo, V.R. (2010) Phillip Ent, 24(1):39-76, Published report
Title:	Variability in response to insecticides of field populations of diamondback moth, *Plutella xylostella* (Linnaeus), in the Philippines
Abstract	Three local populations of the diamondback moth, *Plutella xylostella* (Linnaeus), were collected from crucifer-growing areas in the Philippines were tested for susceptibility to 14 formulated insecticides. The leaf dip technique was used on third larval instar of the pest. The 72-hr LC_{50} values were compared to those of a reference susceptible strain (BRL Sus st). Computed resistance ratios for the three populations showed varied responses in range and levels of resistance to the insecticides. The populations from Atok, Neguet (7400 fasl), Majayjay Laguna (1000 fasl) nad Calamba Laguna (100 fasl) still had very low level of resistance to new insecticide molecules such as flubendiamide, chlorantraniliprole, chlorfluazuron, chlorfenapyr and diafenthiuron, and on the microbial formulation *Bacillus thuringiensis* var *kurstaki*. On the other hand, all three populations showed very high level of resistance to cypermethrin and to malathion. LC_{50} determination for malathion was discontinued when the population showed no mortalities up to 3555 mg/L. For the remaining test insecticides - cartap, indoxocarb, spinosad, fipronil, methamidohpos and teflubenzuron - responses of the reference population vary, ranging from very low to very high level. Discriminating doses (LC_{80} - LC_{90} values) for the formulated insecticides are also proposed as input in development of insecticide resistance monitoring system for DBM.

Evaluation RMS	No remarks

Report:	KMA 3.5/14 - Pereira, S. G.; Sannaveerappanavar, V. T.; Murthy, M. S. (2006) Resistant Pest Management Newsletter, 15(2):26-28, Published report
Title:	Geographical variation in the susceptibility of diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) to *Bacillus thuringiensis* products and acylurea compounds.
Abstract	Field populations of *Plutella xylostella* were collected from Malur, Hassan and Belgaum, Karnataka, India, to study the variation in their susceptibility to *Bacillus thuringiensis* products and acylurea compounds. The treatments comprised: *B. thuringiensis* subsp. *kurstaki*-based products, i.e. Biobit, Delfin and Dipel; and acylurea compounds, i.e. lufenuron and flufenoxuron.

Evaluation RMS	No remarks

Report:	KMA 3.5/15 - Sannaveerappanavar, V.T., Virktamath, C.A. (2006) Resistant Pest Management Newsletter, 15(2):32-35, Published report
Title:	Resistance to insecticides in an Indian strain of diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae)
Abstract	not available

Evaluation RMS	No remarks

Report:	KMA 3.5/16 - Zago, H.B., Siqueira, H.A.A., Pereira, E.J.G., Picanco, M.C., Barros, R. (2014) Pest Management Science, 70(3):488-495, Published report
Title:	Resistance and behavioural Response of *Plutella xylostella* (Lepidoptera: Plutellidae) populations to *Bacillus thuringiensis* formulations
Abstract	BACKGROUND: Insecticide resistance is probably the major cause of control failure of *Plutella xylostella* (L.) in Brazil. In most production regions, the use of chemicals has been the prevalent method of control, with reduced efficacy through cropping seasons, even for the most recent use of products based on *Bacillus thuringiensis* (Bt). The current status of the re-

sistance to these products was assessed, as well as the behavioural response of *P. xylostella* populations to Bt sprays.

RESULTS:

Most populations of *P. xylostella* were resistant to Bt products, particularly to Xentari®WDG (2-54-fold). Differences in walking characteristics of larvae were variable for most populations, for both Dipel®WP and Xentari®WDG, but not associated with resistance. Most females preferred to lay eggs on untreated surfaces and showed a reduced proportion of oviposition on treated surfaces that only correlated with resistance to Dipel®WP ($r = -0.74$, $P = 0.02$).

CONCLUSION:

Broad and indiscriminate use of Bt-based products has selected Brazilian *P. xylostella* populations to resistance. Larval movement appears to be a resistance-independent mechanism. Most populations of *P. xylostella* preferred to lay eggs on Bt-free surfaces, which might be a result of growers' practice of spraying the cabbage head. Reduced oviposition on treated surfaces correlated with physiological resistance, suggesting a behavioural response among the Bt-resistant colonies to Dipel®WP.

Evaluation RMS	No remarks

Report:	KMA 3.5/17 - Franklin, M.T., Ritland, C.E., Myers, J.H. (2010) Molecular Ecology, 19(6):1122-1133, Published report
Title:	Spatial and temporal changes in genetic structure of greenhouse and filed populations of cabbage looper, *Trichoplusia ni*
Abstract	Vegetable greenhouses and field crops. The heated greenhouse environment has altered the natural extinction-recolonization dynamics of *T. ni* populations, and allows year-round persistence in some locations. In addition, the extensive use of the biopesticide, *Bacillus thuringiensis* subspecies *kurstaki* (Bt) in some greenhouses has selected for resistance. Here we investigated the genetic structure of *T. ni* populations in British Columbia greenhouses and in field populations in California and British Columbia using amplified fragment length polymorphisms (AFLP) as related to patterns of Bt resistance. The majority of British Columbia field populations were similar to the California field populations, the potential source of migrants. However, populations in two geographic areas with high concentrations of greenhouses showed local genetic differentiation. Some of these populations experienced severe bottlenecks over-winter and following Bt sprays. Greenhouse populations showed a pattern of isolation by distance and a strong positive relationship between genetic differentiation and levels of Bt resistance. These patterns indicate that greenhouses that sometimes support year-round populations of *T. ni* and the ensuing strong bottlenecking effects following winter clean-ups and Bt application cause genetic differentiation of *T. ni* populations. Long distance migrants to field populations contribute to genetic homogeneity of these.

Evaluation RMS	No remarks

Report:	KMA 3.5/10 - Janmaat, A., Franklin, M., Myers, J.H. (2015) CAB International 2015. Bt resistance (eds. M. Soberón, Y. Gao and A. Bravo), Chapter 5:49-55, Published report
Title:	Resistance of Cabbage Loopers to Btk in a Greenhouse Setting: Occurrence, Spread and Management
Abstract	Resistance to the microbial insecticide Btk occurred in a number of laboratory populations of Lepidoptera before it was discovered in field populations. Therefore, it is not surprising that cabbage loopers (*Trichoplusia ni*) in semi-contained vegetable greenhouses were among the first examples of Btk resistance in agricultural situations. We have studied the occurrence of Btk resistance in cabbage loopers in greenhouse populations and the movement of resistance among greenhouse populations of moths. Migratory populations of cabbage loopers remained highly susceptible to Btk sprays in fields in the vicinity of greenhouses. Complete clean-up of greenhouses to remove any overwintering moths is necessary to reduce selection for resistance. Cabbage loopers represent a model system for

the study of Btk resistance in contained, seasonal environments.

Evaluation RMS	No remarks

Report:	KMA 3.5/18 - Kalia, V., Kumari, A., Mittal, A., Singh, B.P., Nair, R., Gujar, G.T. (2006) Pesticide Research Journal, 18(1):47-50, Published report
Title:	Temporal Variation in susceptibility of American Bollworm, *Helicoverpa armigera* to *Bacillus thuringiensis* (BT) var. *kurstaki* HD-73, ist Cry1Ac Toxin and Bt cotton
Abstract	The temporal susceptibility of neonates of the American bollworm, *Helicoverpa armigera* to *Bacillus thuringiensis* (Bt) var. *kurstaki* HD-73 or its Cry1Ac toxin in the artificial diet assays was investigated for the populations of four different locations. The insect populations, collected post-Bt cotton introduction, showed less susceptibility to Cry1 Ac/HD-73 in the artificial diet assays. The larval susceptibility to Cry1Ac in the cotton leaf dip assays was higher than in the artificial diet assays, possibly due to the synergism of Cry1Ac with cotton leaf allelochemicals. The toxicity of Bt cotton leaves to neonates of *H. armigera* remained high during the course of studies from 2204 – 2000. The absence of resistance to Bt cotton in *H. armigera* is discussed in relation to Bt resistance traits and current management practices.

Evaluation RMS	No remarks

B.3.6 Methods to prevent loss of virulence of seed stock of the micro-organism

Genetic alteration can theoretically occur during production of Bt through mutations or plasmid loss. Therefore, starter cultures all derive from the same original culture. All media and containers are sterilised. The seed stock of the strain is stored as lyophilised culture at -80°C. Under these conditions, genetic alteration is unlikely to occur.

For manufacturing of Btk SA-12 technical material a culture maintenance program is applied to ensure that only genetically unchanged and pure subcultures of the mother culture are used for fermentation. This means that during the production fermentation steps cultures are used which are at most one to two passages away from the original lyophilised stock culture of the strain. Each passage from the original stock culture for preparing working culture lines is subjected to a battery of tests to ensure consistency of the starting material for fermentation. The genetic stability of the strain is ensured and verified by special quality control procedures which include a wide range of methods and procedures. Further information is regarded as confidential. Please refer to Vol. 4, C.1.1.3.

B.3.7 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 6.1.1/07	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

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 3.5/01	Schnepf, E. Crickmore, N. Van Rie, J. Lereclus, D. Baum, J. Feitelson, J. Zeigler, D.R. Dean, D.H.	1998	BACILLUS THURINGINSIS AND ITS PESTICIDAL CRYSTAL PROTEINS Microbiol. Mol. Biol. Rev. 62:775-806 Report no. not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 3.5/02	Tabashnik, B.E.	1994	EVOLUTION OF RESISTANCE TO BACILLUS THURINGINSIS Ann. Rev. Entomol., 39, 47-79 Report-no. not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 3.5/03	Janmaat, A.F., Myers, J.	2003	RAPID EVOLUTION AND THE COST OF RESISTANCE TO BACILLUS THURINGIENSIS IN GREENHOUSE POPULATI-ONS OF CABBAGE LOOPERS, TRICHOPLUSIA NI Proc. Royal Soc. London, 270, 2263-2270 Report-no. not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 3.5/04	Ferré, J., Real, M.D., van Rie, J., Jansens, S., Peferoen, M.	1991	RESISTANCE TO THE BACILLUS THURINGINSIS INSECTICIDE IN A FIELD POPULATION OF PLUTELLA XYLOSTELLA IS DUE TO A CHANGE IN A MIDGUT MEMBRANE RECEPTOR Proc. Natl. Acad. Sci USA, 88, 5119-5123 Report-no. not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 3.5/05	McGaughey, W.H Whalon, M.E.	1992	MANAGING INSECT RESISTANCE TO BACILLUS THURINGINSIS TOXINS Science 258:1451-1455 Report no. not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 3.5/06	Süß, J.	2016	LITERATURE REVIEW ON BACILLUS THURINGINSIS SUBSP. KURSTAKI SA-12 BIOLOGICAL PROPERTIES Certis USA LLC, Report-no.: 2281384-MA-02-01_SA-12 GLP/GEP: no Published: no	no	yes	Protected	CERTIS	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 3.5/07	Wang, L., Li, X.-F., Zhang, J., Zhao, J.-Z., Wu, Q.-J., Xu, B., Zhang, Y.-J.	2007	MONITORING OF RESISTANCE FOR THE DIAMONDBACK MOTH TO BACILLUS THURINGIENSIS Cry1Ac AND Cry1Ba TOXINS AND A BT COMMERCIAL FORMULATION Journal of Applied Entomology, 131(7), 441-446 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated
KMA 3.5/08	Mittal, A., Kumari, A., Kalia, V., Kumar Singh, D., Gujar, G.T.	2007	SPATIAL AND TEMPORAL BASELINE SUSCEPTIBILITY OF DIAMONDBACK MOTH, PLUTELLA XYLOSTELLA (LINNAEUS) TO BACILLUS THURINGIENSIS SPORE CRYSTAL MIXTURE, PURIFIED CRYSTAL TOXINS AND MIXTURES OF cry TOXINS IN INDIA Biopesticides International, 3(1), 58-70 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated
KMA 3.5/09	Gong, Y., Wang, C., Yang, Y., Wu, S., Wu, Y.	2010	CHARACTERIZATION OF RESISTANCE TO BACILLUS THURINGIENSIS TOXIN Cry1Ac IN PLUTELLA XYLOSTELLA FROM CHINA Journal of Invertebrate Pathology, 104(2), 90-96 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated
KMA 3.5/10	Janmaat, A., Franklin, M., Myers, J.H.	2015	RESISTANCE OF CABBAGE LOOPERS TO BTK IN A GREENHOUSE SETTING: OCCURRENCE, SPREAD AND MANAGEMENT Bt Resistance (M. Soberón, T. Gao and A. Bravo, edt.), 5:49-55 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated
KMA 3.5/11	Jiang, T., Wu., S., Yang, T., Zhu, C., Gao, C.	2015	MONITORING FIELD POPULATIONS OF PLUTELLA XYLOSTELLA (LEPIDOPTERA: PLUTELLIDAE) FOR RESISTANCE TO EIGHT INSECTICIDES IN CHINA Florida Entomologist, 98(1), 65-73 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 3.5/12	Xia, Y., Lu, Y., Shen, J., Gao, X., Qiu, H., Li, J.	2014	RESISTANCE MONITORING FOR EIGHT INSECTICIDES IN PLUTELLA XYLOSTELLA IN CENTRAL CHINA Crop Protection, 63, 131-137 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated
KMA 3.5/13	Sarmiento, G.M., Ocampo, V.R.	2010	VARIABILITY IN RESPONSE TO INSECTICIDES OF FIELD POPULATIONS OF DIAMONDBACK MOTH, PLUTELLA XYLOSTELLA (LINNAEUS), IN THE PHILIPPINES Philipp. Ent., 24(1), 39-76 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated
KMA 3.5/14	Pereira, S. G., Sannaveerappanavar, V. T., Murthy, M. S.	2006	GEOGRAPHICAL VARIATION IN THE SUSCEPTIBILITY OF DIAMONDBACK MOTH, PLUTELLA XYLOSTELLA (L.) (LEPIDOPTERA: YPONOMEUTIDAE) TO BACILLUS THURINGINSIS PRODUCTS AND ACYLUREA COPOUNDS Resistant Pest Management Newsletter, 15(2), 26-28 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated
KMA 3.5/15	Sannaveerappanavar, V.T., Virktamath, C.A.	2006	RESISTANCE TO INSECTICIDES IN AN INDIAN STRAIN OF DIAMONDBACK MOTH, PLUTELLA XYLO-STELLA (L.) (LEPIDOPTERA: YPONOMEUTIDAE) Resistant Pest Management Newsletter, 15(2), 32-35 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated
KMA 3.5/16	Zago, H.B., Siqueira, H.A.A., Pereira, E.J.G., Picanco, M.C., Barros, R.	2014	RESISTANCE AND BEHAVIOURAL RESPONSE OF PLUTELLA XYLOSTELLA (LEPIDOPTERA: PLUTELLIDAE) POPULATIONS TO BACILLUS THURINGIENSIS FORMULATIONS Pest Management Science, 70(3), 488-495 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 3.5/17	Franklin, M., Ritland, C.E., Myers, J.H.	2010	SPATIAL AND TEMPORAL CHANGES IN GENETIC STRUCTURE OF GREEN-HOUSE AND FIELD POPU-LATIONS OF CAGGAGE LOOPER, TRICHOPLUSIA NI Mol. Ecol., 19(6), 1122-1133 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated
KMA 3.5/18	Kalia, V., Kumari, A., Mittal, A., Singh, B.P., Nair, R., Gujar, G.T.	2006	TEMPORAL VARIATION IN SUSCEPTIBILITY OF AMERICAN BOLLWORM, HELICOVERPA ARMIGERA TO BACILLUS THURINGIN-SIS (BT) VAR. KURSTAKI HD-73, IST Cry1Ac TOXIN AND BT COTTON Pesticide Research Journal, 18(1), 47-50 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated