# *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 (PPP) – CoStar WG

**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 Summary, evaluation and assessment of the data and information

# 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 11 July 2008 a draft review report to the Standing Committee on the Food Chain and Animal Health, for final examination. The draft review report was finalized in the meeting of the Standing Committee on 11 July 2008. Subsequently Regulation (EC) No 1107/2009 repealed and replaced Directive 91/414/EEC and the active substance Btk SA-12, was deemed to be approved under that Regulation and included in the Annex to Regulation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.

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 $5.7 \times 10^{13}$ 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 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.

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 information" 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. There might be some exceptions but in this case justifications/explanations are provided.

Representative uses chosen for renewal of Btk SA-12 cover control of *Cydia pomonella* in pome fruits and *Spodoptera* spp. in ornamentals 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 / $1.35 \times 10^{11}$ b) 7650 / $8.1 \times 10^{11}$ | 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 / $9.0 \times 10^{10}$ b) 5100 / $5.4 \times 10^{11}$ | 200-1000 |
| Ornamentals | F | *Spodoptera* spp. | Foliar spray | BBCH 12-89 | a) 6 (7) b) 6 (7) | a) 2.0 b) 12.0 | a) 1700 / $1.8 \times 10^{11}$ b) 10200 / $1.1 \times 10^{12}$ | 500-1000 |

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

# B.9 Effects on non-target organisms

## B.9.1 Effects on birds and mammals

### B.9.1.1 Toxicity, invectiveness and pathogenicity in birds and mammals

**Effects on birds and mammals**

As CoStar WG was not the representative formulation for original approval of Btk SA-12, no data on the formulation have been submitted before. According to the EFSA peer review of the risk assessment of the five Btk strains[1] the extrapolation between different *Bacillus thuringiensis kurstaki* strains can be considered acceptable for non-target organisms, except for daphnids and non-target arthropods.

**New data 2016**

No study assessing the effect of CoStar WG on birds is submitted here. It is referred to the information submitted for the active substance *Bacillus thuringiensis* subsp. *kurstaki* strain SA-12 in Vol. 3 MA, Section 9, Point B.9.1.1.

The ingredients of the preparation CoStar WG, formulated as water dispersible granule, are inert and no hazards to the environment are expected (please refer to Volume 4). Therefore, studies and information on the microbial pest control agent, *Bacillus thuringiensis* subsp. *kurstaki* SA-12, are considered applicable and relevant with regard to the evaluation of effects on birds of the formulated products.

**Toxicity**

Birds

No strain specific data for Btk SA-12 were submitted but the short-term toxicity of Btk EG2348 and the technical Btk SA-11 to *Colinus virginianus* was evaluated in the dRAR for Btk SA-11 and Btk EG2348, respectively (please refer Volume 3 MA, Section B.9, Point B.9.1 for Btk SA-11 and Btk EG2348, respectively). The test substance was administered at a daily dose of 3333 mg/kg bw/day or 22 mg/kg bw/day respectively for five days in both studies. No treatment related mortalities or effects of Btk occurred in the test organism. The acute $LD_{50}$ can be determined to lie above the tested concentration of 3333 mg/kg bw. Since Btk EG2348 caused no signs of toxicity or pathogenicity at the highest tested concentration (3333 mg/kg bw) and due to the high similarity of Btk SA-11, EG2348 and Btk SA-12 it is assumed that the $LC_{50}$ value of 3333 mg/kg bw is also applicable for Btk SA-12. Furthermore, a study to determine the oral pathogenicity and acute oral toxicity of Thuricide SC (liquid formulation of Btk SA-12) is submitted (please refer to Vol. 3 MA, Section B.9, Point B.9.1/01). In this study, no signs of toxicity or mortality were observed after 30 days and the acute oral $LC_{50}$ was estimated to be > $5.0 \times 10^9$ CFU/kg bw.

Mammals

Several acute oral toxicity studies on mammals with strains of Btk SA-11, SA-12 and Btk EG2348 have been conducted. Please refer Volume 3 MA, Section B.6, for Btk SA-11, Btk SA-12 and Btk EG2348, respectively. One study investigated the effects of an oral gavage of *Bacillus thuringiensis* SA-12 to Sprague-Dawley rats (please refer to the dRAR, Volume 3 MA, Section B.6, for Btk SA-12). No test substance related signs of infectivity were observed in the study, so that the acute oral $LD_{50}$ was estimated to be > $5.4 \times 10^8$ CFU/animal corresponding to $2.7 \times 10^9$ CFU/kg bw (if a body weight of 200 g is considered).

In a similar study (please refer to the dRAR, Vol. 3 MA, Section B.6, for Btk SA-12, and EFSA Journal 2012;10(2):2540[1]), the $LD_{50}$ for Sprague-Dawley rats the acute $LD_{50}$ was determined to be > 5050 mg test substance/kg bw.

Relevant endpoints from studies with birds and mammals are summarised in Table 9.1.1-1.

[1] 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

**Table 9.1.1-1 Summary of the studies on effects on birds and mammals; toxicity and pathogenicity of *B. thuringiensis subsp. kurstaki***

| Test substance | Test species | Endpoint* | Reference |
|---|---|---|---|
| *B. thuringiensis subsp. kurstaki* (EG2348) | Bobwhite quail | The risk to birds is assessed as low. **$LD_{50}$ (Btk) > 3333 mg MPCA/kg bw** (only stated as mg/kg) | Vol. 3 MA, Section B.9, Point B.9.1 for Btk EG2348 & EFSA Journal 2012;10(2):2540[2] |
| SAN 415 I Technical (SA-11) | Bobwhite quail | 5-d $LD_{50}$ > $3.86 \times 10^9$ CFU/kg bw (corresp. to 22 mg a.s./kg bw/day) | Vol. 3 MA, Section B.9, Point B.9.1 for Btk SA-11 |
| Thuricide SC (SA-12) | Japanese quail | **30-d $LD_{50}$ > $5.0 \times 10^9$ CFU/kg bw** | Vol. 3 MA, Section B.9, Point B.9.1 for Btk SA-12 |
| *B. thuringiensis subsp. kurstaki* (SA-12) | Rat, acute oral | The risk to mammals is assessed as low. $LD_{50}$ > 5050 MPCP mg/kg bw **($LD_{50}$ > $2 \times 10^{11}$ CFU/kg bw)** | EFSA Journal 2012;10(2):2540[3] |
| *B. thuringiensis subsp. kurstaki (*SA-12) | Rat, acute oral | $LD_{50}$ > $5.9 \times 10^8$ CFU/kg bw | Vol. 3 MA, Section B.6, Point B.6.3. for Btk SA-12 |
| Thuricide SC (SA-12) | Rat, acute oral | $LD_{50}$ > $5.4 \times 10^8$ CFU/animal corresponding to $2.7 \times 10^9$ CFU/kg bw | Vol. 3 MA, Section B.6, Point B.6.3. for Btk SA-12 |
| SAN 415 WG formulation (SA-11) (Javelin-R/Delfin-R) (SA-11) | Rat, oral / 14-day observation | $LD_{50}$ > 5 g Delfin /kg bw ($LD_{50}$ > $1.12 \times 10^{11}$ CFU/kg bw) (corresp. to 4250 mg a.s./kg bw) | Vol. 3 MA, Section B.6, Point B.6.3 for Btk SA-11 |
| Delfin WG (SA-11) | Mouse, oral / 14-day observation | $LD_{50}$ > 5 g Delfin WG/kg bw ($LD_{50}$ > $2.4 \times 10^{11}$ CFU/ kg bw) (corresp. to 4250 mg a.s./kg bw) | Vol. 3 MA, Section B.6, Point B.6.3 for Btk SA-11 |

* Endpoints marked in **bold** are used for the risk assessment

The available endpoints for birds and mammals indicate no toxicity or pathogenicity of any of the different strains of *Bacillus thuringiensis* subsp. *kurstaki* independently of the study design. No effects on birds and mammals have been reported. Nevertheless, the risk assessment will be based on the most feasible endpoints available considering all strains.

## B.9.1.2 Risk assessment for birds and mammals

**Exposure**

Birds and mammals are typically exposed to dry residues on their food items following the dilution and via drinking water following spraying of the formulated product. During spraying, much of the formulation constituents are likely to be lost by volatilisation. Therefore, where oral exposure is the main route of exposure, toxicity data for the active substance are used in preference to data from tests with the formulated material. Exposure via dermal and inhalation routes is considered unlikely, since at the time of application and for a short period thereafter, most wild birds and mammals will leave the immediate vicinity of spray operations in response to the human disturbance. Birds and mammals may be exposed directly and indirectly via the ingestion of sprayed plant parts and via infected arthropods, respectively.

[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

The potential exposure of birds to Btk was estimated following GAP directed applications of the product in the different uses at maximum application rates.

**Risk Assessment - Birds and Mammals**

For risk assessment for effects on birds and mammals the 'European Food Safety Authority Guidance Document on Risk Assessment for Birds and Mammals' (EFSA Guidance document 2009)[3] is available. However, this document in first line is compiled for the risk assessment of chemical substances. Therefore, the risk assessment approach is not feasible for microbial substances as not only biological parameters of the birds and mammals go into calculations but also chemical properties, like $K_{oc}$ values from the test item, 90$^{th}$ percentile residue values that come from a database for chemicals.

For the exposure via drinking water a risk assessment in accordance to SANCO 4145/2000[4] is presented, which is considered more appropriate and is considered to represent a worst-case. For the exposure of birds and mammals via food intake a first tier calculation according to EFSA guidance is also presented, however, this approach should be considered indicative only.

**Exposure via drinking water**

Risk assessment for drinking water is performed in accordance with SANCO 4145/2000[5]. Species that frequent open water bodies are able to ingest residues of active substances that reach water for example via spray drift from treated fields. The exposure density in this case is equal to $PED_{sw}$, calculated under Volume 3 MP, Section B.8, Point B.8.1.2 for Btk SA-12.

In some situations, some species may obtain all their daily water demand directly from puddles of spray liquid or reservoirs held in the axils of leaves. This situation can be considered as worst case. The exposure density can be calculated from the dilution used to prepare the product for spraying. Analysis has shown that initial densities in such sources are in the range 5 - 20% of the sprayed concentration, therefore a dilution factor of 5 is applied for the risk assessment.

Thus the $PED_{puddle}$ is calculated as:

$PED_{puddle}$ = maximum spray solution density × 0.20

The daily water intake is calculated as follows:

Birds: Total water ingestion rate (L/day) = $0.059 \times W^{0.67}$

Mammals: Total water ingestion rate (L/day) = $0.099 \times W^{0.9}$

Where:

W = body weight in kg

Thus, the daily dose of active substance intake is calculated as

$$\text{Daily dose} = \frac{PED_{puddle} \times \text{total water ingestion rate}}{W}$$

Where:

W = body weight in kg

The risk of *Bacillus thuringiensis* subsp. *kurstaki* SA-12 to birds and mammals was assessed from margin of safety (MOS; corresponding to TER) values according to the following equation:

$$MOS = \frac{LD_{50}[\text{CFU/kg bw}]}{\text{daily dose}[\text{CFU/kg bw}]}$$

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

[4] European Commision, Health & Consumer Protection Directory, Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC, SANCO/4145/2000 - final, 25 September 2002

Based on the available data the MOS values of birds and mammals for Btk SA-12 were calculated **(**Table 9.1.2-1**)**

**Table 9.1.2-1 Risk assessment for birds and mammals for exposure via drinking water (puddles) following GAP directed application of CoStar WG in ornamentals in accordance with SANCO 4145/2000[5]**

| Indicator species[1)] | Body weight [kg] | Total water ingestion rate [L/day] | maximum spray solution concentration (ornamentals) [CFU/L] | $PED_{puddle}$ [CFU/L] | Daily dose [CFU/kg bw] | Toxicity [a)] $LD_{50}$ [CFU/kg bw] | MOS |
|---|---|---|---|---|---|---|---|
| Small herbivorous mammal - vole | 0.025 | 0.003579 | $2.28 \times 10^{11}$ | $4.56 \times 10^{10}$ | $6.53 \times 10^{9}$ | $> 2.0 \times 10^{11}$ | > 30.6 |
| Insectivorous bird - tit, wren | 0.010 | 0.002697 | | | $1.23 \times 10^{10}$ | $> 5.0 \times 10^{9}$ | > 0.41 |

1) In the SANCO 4145/2000[5] no scenario for ornamentals is provided. However, in accordance with EFSA Guidance document 2009[6] small herbivorous mammal and small insectivorous bird are indicator species in the acute risk assessment screening step for ornamentals

a) The presented $LD_{50}$ are "greater than" values. No lethal, sublethal or pathogenic effects have been observed at these highest rates tested.

Calculation of the exposure via water can be considered worst case. The density in the water is directly related to the spray application. In the drinking water risk assessment for birds and mammals the SA-12 strain specific endpoints in CFU/kg bw are used for the calculations. The resulting MOS of > 0.41 for insectivorous birds indicates a potential risk for the indicator species tit/wren. However, all presented $LD_{50}$ are "greater than" values. No lethal, sublethal or pathogenic effects have been observed at these highest rates tested. The EU agreed endpoint for *B. thuringiensis subsp. kurstaki* based on a study with Btk strain EG2348 is with 3333 mg/kg bw about a factor 1000 higher than the SA-12 strain specific endpoint used. Due to the similarity of the different strains it can be concluded that the toxicity and pathogenicity of the different strains are of a comparable magnitude. Taking into consideration the absence of effects on birds and mammals at concentration higher than the worst case exposure, no adverse effects in birds and mammals are to be expected due to exposure to contaminated drinking water.

Birds and mammals may be exposed to CoStar WG also by feeding on sprayed vegetation, seeds or insects. Standard exposure scenarios for the intended uses are therein described (please refer to the **Critical GAP provided in the introduction** for details). The risk for indicator species of each scenario was assessed in a screening assessment. Data on short-term toxicity are used as they cover acute toxicity to birds and mammals.

In line with the recommendations in the EFSA Guidance document (2009)[7], the daily dietary dose (DDD) was calculated for the active substance with the following formula:

DDD (multiple) = application rate (kg/ha) × shortcut value

With:

Shortcut value = default parameter combining food intake rate, body weight, concentration of the substance in the diet (based on the 90$^{th}$ percentile residues) and the fraction of diet obtained in the treated area for the bird indicator species/crop combination in question. It must be kept in mind that residue data were measured for chemical substances and therefore the approach should be considered indicative only.

[5] European Commision, Health & Consumer Protection Directory, Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC, SANCO/4145/2000 - final, 25 September 2002

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

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

Though multiple applications are foreseen, a corresponding MAF (multiple application factor) is not considered. Due to the proven instability of *B. thuringiensis* spores when exposed to sunlight, the application of a MAF is not considered feasible. Furthermore, the MAF was established for chemical substances and it is not sure that the MAF as from the guidance can reflect the degradation of spores.

The MOS value was calculated by dividing the acute endpoint by the daily dietary dose (DDD) for each application rate. The screening assessments for both, birds and mammals, are shown in **Table** 9.1.2-2 and **Table** 9.1.2-3.

**Table 9.1.2-2 Screening assessment for birds following GAP directed application of CoStar WG**

| Indicator species | Crop | Toxicity $LD_{50}$ | Application rate [kg a.s./ha] | MAF | Short cut value[a)] | DDD | MOS |
|---|---|---|---|---|---|---|---|
| Small insectivorous birds | Orchards | > 3333 mg/kg bw | 1.275 | Not justified | 46.8 | 59.7 | > 55.8 |
| | Ornamentals | | 1.7 | Not justified | 46.8 | 79.6 | > 41.9 |

[a)] Short cut value based on the 90th percentile of residues provided in EFSA Guidance document (2009)[7]

**Table 9.1.2-3 Screening assessment for mammals following GAP directed application of CoStar WG**

| Indicator species | Crop | Toxicity $LD_{50}$ | Application rate [kg a.s./ha] | MAF | Short cut value[a)] | DDD | MOS |
|---|---|---|---|---|---|---|---|
| Small herbivorous mammals | Orchards | > 4250 mg/kg bw | 1.275 | Not justified | 136.4 | 173.9 | > 24.4 |
| | Ornamentals | | 1.7 | Not justified | 136.4 | 231.9 | > 18.3 |

[a)] Short cut value based on the 90th percentile of residues provided in EFSA Guidance document (2009)[7]

Based on the acute toxicity data the calculated margin of safety values are determined to be large enough for birds, and mammals for all uses. Concurrently to the shortcomings of the use of EFSA Guidance document (2009)[7] for the assessment of microorganisms already mentioned above, the value is based on the highest tested dose from three studies where no adverse effects were observed, thus leading to an over-exaggeration of any theoretical risk. Moreover, exposition to *B. thuringiensis* is also overestimated, as the calculation does not take into account the rapid inactivation of *B. thuringiensis* in the absence of host insect. Furthermore, in the environment, small mammals are constantly exposed to *B. thuringiensis* spores, as this is a naturally occurring soil bacteria and application only represents a transient shift in population density.

According to the Commission Regulation (EU) No 546/2001, Part II, Uniform principles for evaluation and authorisation of plant protection products containing micro-organisms[8], Part B article 2.8.1.1., a micro-organism may give rise to risks because of its potential to infect and multiply in avian and mammalian host systems. Whether or not identified risks could be changed due to the formulation of the plant protection product shall be assessed, taking into account the following information on the micro-organism:

(a) its mode of action,

(b) other biological properties,

(c) studies on mammalian toxicity, pathogenicity and infectivity,

(d) studies on avian toxicity, pathogenicity and infectivity.

Also in Commission Regulation (EU) No 546/2001, Part II, Uniform principles for evaluation and authorisation of plant protection products containing micro-organisms[8], Part C article 2.8.1., where there is a possibility of birds and other non-target terrestrial vertebrates being exposed, no authorisation shall be granted if:

[8] Commission Regulation (EU) No 546/2011: Uniform Principles for Evaluation and Authorisation of Plant Protection Products, as provided for in Article 29(6) of Regulation (EC) No 1107/2009

(a) the micro-organism is pathogenic to birds and other non-target terrestrial vertebrates,

(b) in case of toxic effects due to components in the plant protection product, such as relevant metabolites/toxins, the toxicity/exposure ratio is less than 10 on the basis of the acute $LD_{50}$ value or the long-term toxicity/exposure ratio is less than 5, unless it is clearly established through an appropriate risk assessment that under field conditions no unacceptable effects occur - directly or indirectly - after use of the plant protection product according to the proposed conditions of use.

Therefore, based on the mode of action to their insect host, *Bacillus thuringiensis* subsp. *kurstaki* SA-12 and the biological properties of the natural occurring organism, an acceptable risk to birds and mammals is expected. In the available studies no toxicity, infectivity and pathogenicity is reported at the highest tested dose level and *Bacillus thuringiensis* subsp. *kurstaki* SA-12 is of low pathogenicity to birds and other non-target terrestrial vertebrates. No toxic effects were seen in any of the available studies and therefore the calculation of toxicity/exposure ratio is less relevant.

No short- or long-term effects are to be anticipated. Sensitivity to low pH values encountered in the stomach of both birds and mammals renders survival and colonisation of the interior via ingestion unlikely. Based on all available information combined with the available exposure assessments the risk to birds and mammals is considered acceptable.

| | |
|---|---|
| RMS evaluation | No toxic effects were seen in any of the available studies. No short- or long-term effects are to be anticipated. Sensitivity to low pH values encountered in the stomach of birds and mammals renders survival and colonisation of the animals' interior via ingestion unlikely. The literature search covering the last 10 years and focussing to target possible toxicity or pathogenicity of Btk to birds and mammals did not provide any relevant information indicating a potential risk for birds or mammals in spite of the worldwide use of *B. thuringiensis* subsp. *kurstaki* SA-12 as well as other strains belonging to the subspecies.<br><br>Based on the mode of action to their insect host, *Bacillus thuringiensis* subsp. *kurstaki* SA-12 and the biological properties of the natural occurring organism, no risk to birds and mammals are expected. Btk SA-12, like other approved Btk strains, 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. In the available studies no toxicity, infectivity and pathogenicity is reported at the highest tested dose level.<br><br>Risk assessment for birds and mammals for exposure via drinking water and food intake according to EFSA guidance are presented above. It must be kept in mind that guidance is developed for chemical substances and shortcomings for the assessment of microorganisms are significant. For the assessment of microorganisms the value is based on the highest tested dose from studies where no adverse effects were observed, thus leading to an over-exaggeration of any theoretical risk. Therefore, the approach should be considered indicative only.<br><br>In conclusion the proposed use of CoStar WG does not pose an unacceptable risk to birds or mammals. |

## B.9.2 Effects on aquatic organisms

As CoStar WG was not the representative formulation for original approval of Btk SA-12 no data on the formulation have been submitted before. According to the EFSA peer review of the risk assessment of the five Btk strains[1] the extrapolation between different *Bacillus thuringiensis kurstaki* strains can be considered acceptable for non-target organisms, except for daphnids and non-target arthropods.

**New data 2016**

It is referred to the information submitted for the MA *Bacillus thuringiensis* subsp. *kurstaki* SA-12 in Vol. 3 MA, Section B.9, Point B.9.2.

In addition, three studies are presented here assessing the effects of CoStar WG on aquatic organisms - *Oncorhynchus mykiss*, *Daphnia magna* and the green algae *Desmodesmus subspicatus.*

## B.9.2.1 Effects on fish

| | |
|---|---|
| Report: | KMP 9.2.1/01 - [redacted](2010) |
| Title: | CoStar- Acute toxicity testing in Rainbow trout (*Oncorhynchus mykiss*) (Teleostei, Salmonidae) |
| Document No: | Report No. S10-02547 |
| Guidelines: | OECD Guideline No 203 (1992) |
| GLP | Yes |
| Validity | Yes |

**Executive summary**

In a 96-h acute toxicity study, rainbow trout (*Oncorhynchus mykiss*) were exposed to CoStar WG at nominal concentrations of 0 (control) and 100 mg/L under static conditions.

No fish died during the test in the control and in the test concentration. No lethal or sublethal effects were observed.

The 96-h $LC_{50}$ was determined to be above 100 mg/L (nominal) and 51 mg/L (actual) with a probability of 99% according to OECD 203. The NOEC (96 h) was observed at 100 mg/L (nominal) and 51 mg/L (actual).

**MATERIAL AND METHODS**

**Test Item**

| | |
|---|---|
| Designation | CoStar WG |
| Active ingredient | *Bacillus thuringiensis kurstaki* strain SA-12 |
| Characteristics | Brown granular |
| Batch no. | 7106 |
| Expiration date | 08.06.2011 |
| Purity | $8.9 \times 10^{12}$ cfu/kg (nominal); $9.26 \times 10^{12}$ cfu/kg (actual) |

**Test System**

| | |
|---|---|
| Species | Rainbow trout (*Oncorhynchus mykiss*) (Walbaum) |
| Source | [redacted] |
| Number | Control group: 7, Treated group: 7 |
| Weight | Not specified |
| Length | From 4 to 6 cm |
| Acclimatisation period | More than 12 days under test conditions |
| Food | Granular rearing food (size 0) with approx. 2% of their body weight |

**Test Conditions**

| | |
|---|---|
| Temperature | From 15.1 to 16.6°C |
| Photoperiod | 12 - 16 hour photoperiod daily |
| Oxygen content | ≥ 90% of air saturation |
| Hardness | 13°dH |
| pH | from 7.97 to 8.48 |

**Study Design and Methods**

| | |
|---|---|
| In-life dates | 13.07.2010 to 17.07.2010 |
| System | Static |
| Duration | 96 hours |
| Test vessel | 25-Litre capacity containers |
| Concentration | 0 (Control) and 100 mg/L |
| Observations | Fish were observed at 3, 6, 24, 48, 72 and 96 hours for mortality, occurrence of visible abnormalities (loss of equilibrium, swimming behaviour, respiratory function, pigmentation), dissolved oxygen, pH and temperature |

**RESULTS AND DISCUSSION**

No mortality was occurred during the 96-hour period in the control and in the test concentration. No lethal or sub-lethal effects were observed.

**CONCLUSIONS**

The 96-h $LC_{50}$ was determined to be above 100 mg/L (nominal) and 51 mg/L (actual, based on CFU counts in the test solution) with a probability of 99% according to OECD 203. The NOEC (96 h) was observed at 100 mg/L (nominal) and 51 mg/L (actual) corresponding to $4.7\times 10^8$ CFU/L.

**Toxic effects / Infectivity / Pathogenicity of plant protection product to fish**

| | |
|---|---|
| Test species | Rainbow trout (*Oncorhynchus mykiss*) |
| Toxicity of plant protection product | Effects on fish 96-hour (static limit): $LC_{50}$ >51 mg /L |
| Infectivity / Pathogenicity | The duration of the test was too short to account for any infectivity and pathogenicity. |

**Comments and conclusion RMS:**

The test was performed according to OECD 203. The validity criteria according to this guideline were fulfilled.
Validity criteria OECD 203 (fish):

- No dead fish in untreated control – met
- Oxygen saturation > 60% - met

The study is considered acceptable. In a 96-h acute toxicity study, rainbow trout (*Oncorhynchus mykiss*) were exposed to CoStar WG at nominal concentrations of 0 (control) and 100 mg/L under static conditions. No mortality or adverse effects were observed in the fish.

The 96-h $LC_{50}$ was determined to be above 100 mg/L (nominal) and 51 mg/L (actual) with a probability of 99% according to OECD 203. The NOEC (96 h) was observed at 100 mg/L (nominal) and 51 mg/L (actual).

The duration of the test was too short to account for any infectivity and pathogenicity. It can be concluded that under the condition of this test the product is not toxic to fish.

Endpoint:
Effects on fish 96-hour (static limit): $LC_{50}$ >51 mg /L corresponding to $4.7\times 10^8$ CFU/L, based on the actual CFU measured in the test solutions.

## B.9.2.2 Effects on freshwater invertebrates

| | |
|---|---|
| Report: | KMP 9.2.2/01 - Dengler, D. (2010) |
| Title: | Assessment of toxic effects of CoStar on *Daphnia magna* using the 48 h acute immobilisation test |
| Document No: | Report No. S10-02549 |
| Guidelines: | OECD Guideline No. 202 (2004) |
| GLP | Yes |
| Validity | Yes |

**Executive summary**

In a 48-h acute immobilisation test, *Daphnia magna* were exposed to CoStar WG at nominal concentrations of 0 (control) and 100 mg/L under static conditions.

After 24 and 48 hours of exposure, no immobile daphnids were observed in the control and at 100 mg/L.

The 48-h $EC_{50}$ was determined to be above 100 mg/L (nominal) and above 141 mg/L (actual). The NOEC (48 h) was observed at 100 mg/L (nominal) and 141 mg/L (actual).

**MATERIAL AND METHODS**

**Test Item**

| | |
|---|---|
| Designation | CoStar WG |
| Active ingredient | *Bacillus thuringiensis kurstaki* strain SA-12 |
| Characteristics | Brown granular |
| Batch no. | 7106 |
| Expiration date | 08.06.2011 |
| Purity | $8.9 \times 10^{12}$ cfu/kg (nominal); $9.26 \times 10^{12}$ cfu/kg (actual) |

**Test System**

| | |
|---|---|
| Species | *Daphnia magna* Straus, clone V |
| Source | Rearing stock at the testing facility |
| Number | Control group: 20, Treated group: 20 |
| Food | Single cell green algae (*Desmodesmus subspicatus*) |

**Test Conditions**

| | |
|---|---|
| Temperature | From 20.0 to 21.5°C |
| Photoperiod | 16 hour photoperiod daily (~1104 lux) |
| Oxygen content | ≥ 64% of air saturation |
| Hardness | 11°dH (196.33 mg/L as $CaCO_3$) |
| pH | from 7.92 to 8.36 |

**Study Design and Methods**

| | |
|---|---|
| In-life dates | 07.07.2010 to 09.07.2010 |
| System | Static |
| Duration | 48 hours |
| Test vessel | 100 mL glass beaker |
| Concentration | 0 (Control) and 100 mg/L |

| | |
|---|---|
| | 4 replicates per group |
| Observations | After 24 and 48 hours the immobilised daphnids were counted. |

**RESULTS AND DISCUSSION**

After 24 and 48 hours of exposure, no immobile daphnids were observed in the control and at 100 mg/L.

**CONCLUSIONS**

The 48-h $EC_{50}$ was determined to be above 100 mg/L (nominal) and above 141 mg/L (actual, based on CFU counts in the test solution). The NOEC (48 h) was observed at 100 mg/L (nominal) and 141 mg/L (actual) corresponding to $1.3 \times 10^9$ CFU/L.

**Toxic effects / Infectivity / Pathogenicity of plant protection product to freshwater invertebrates**

| | |
|---|---|
| Test species | *Daphnia magna* |
| Toxicity of plant protection product | Effects on daphnia: 48-hour (static limit): $EC_{50}$ > 141 mg/L |
| Infectivity / Pathogenicity | The duration of the test was too short to account for any infectivity and pathogenicity. |

**Comments and conclusion RMS:**

The test was performed according to OECD 202. The validity criteria according to this guideline were fulfilled.
Validity criteria OECD 202 (daphnids):

- Not more than 10% of immobilised daphnids in untreated control – met
- Oxygen saturation > 30% - met

The study is considered acceptable. In a 48-h acute immobilisation test, *Daphnia magna* were exposed to CoStar WG at nominal concentrations of 0 (control) and 100 mg/L under static conditions.

After 24 and 48 hours of exposure, no immobile daphnids were observed in the control and at 100 mg/L. The 48-h $EC_{50}$ was determined to be above 100 mg/L (nominal) and above 141 mg/L (actual).

The duration of the test was too short to account for any infectivity and pathogenicity. It can be concluded that under the condition of this test the product is not toxic to daphnids. The $LC_{50} > 1.3 \times 10^9$ CFU/L, based on the measured CFU in the test solution.

Endpoint:
Effects on daphnia: 48-hour (static limit): $EC_{50}$ > 141 mg/L corresponding to $1.3 \times 10^9$ CFU

## B.9.2.3 Effects on algae growth

| | |
|---|---|
| Report: | KMP 9.2.3/01 - Weber, K. (2011) |
| Title: | Testing of effects of CoStar to the single cell green alga *Desmodesmus subspicatus* |
| Document No: | Report No. S10-02550 |
| Guidelines: | OECD Guideline No. 201 (2006) |
| GLP | Yes |
| Validity | yes |

**Executive summary**

In a 72-h growth inhibition test, *Desmodesmus subspicatus* were exposed to CoStar WG at nominal concentrations of 0 (control) and 18.8, 37.5, 75.0, 150 and 300 mg/L. The average specific growth rate and the percentage of growth rates were determined on day 1, 2 and 3, and the yield was calculated.

No statistical inhibitory effects on growth rate and yield were observed on day 3 up to the highest test concentration of 300 mg/L.

No statistical significant difference from the control was determined up to 300 mg/L on day 3. Therefore, the NOEC is determined to be 300 mg/L (nominal) and 696 mg/L (actual); and the LOEC is estimated to be > 300 mg/L (nominal) and > 696 mg/L (actual). The $E_rC_{50}$ and $E_yC_{50}$ could not be determined since the inhibition was not significant and below 50%.

## MATERIAL AND METHODS

### Test Item

| | |
|---|---|
| Designation | CoStar WG |
| Active ingredient | *Bacillus thuringiensis kurstaki* strain SA-12 |
| Characteristics | Brown granular |
| Batch no. | 7106 |
| Expiration date | 08.06.2011 |
| Purity | $8.9 \times 10^{12}$ cfu/kg (nominal); $2.15 \times 10^{13}$ cfu/kg (actual) |

### Test System

| | |
|---|---|
| Species | *Desmodesmus subspicatus* SAG strain no. 86-81 |
| Source | Rearing stock at the testing facility |

### Test Conditions

| | |
|---|---|
| Temperature | From 23 to 25°C |
| Photoperiod | Continuous light 4000-6000 lux) |
| pH | from 7.36 to 7.78 (test start) and 8.59 to 10.34 (test end) |

### Study Design and Methods

| | |
|---|---|
| In-life dates | 29.06.2010 to 10.12.2010 |
| Duration | 72 hours |
| Test vessel | 500 mL glass beaker inoculated with $0.5 \times 10^4$ cells/mL at test start |
| Concentration | 0 (Control) and 18.8, 37.5, 75.0, 150 and 300 mg/L<br>6 replicates for the control; 3 replicates for the test concentrations. |
| Experimental treatment | The algae were exposed to different concentrations of the test item under defined conditions in a synthetic growth medium during several generations. By comparing the cell division under test conditions with and without the influence of the test item, an inhibition of the cell multiplication is calculated. This inhibition is a value for toxicity. The cell numbers were determined by fluorescence detection. |
| Evaluation and calculation of inhibitory effects | At defined days (day 1, 2 and 3) the number of cells in each replicate was evaluated. The concentration which resulted in 50% inhibition of cell growth rate ($E_rC_{50}$), the concentration which did not yet cause any inhibition (NOEC) and the lowest observed effect concentration (LOEC) were determined. Additionally, the concentration bringing about 50% inhibition of yield ($E_yC_{50}$) was determined. |

## RESULTS AND DISCUSSION

No statistical inhibitory effects on growth rate and yield were observed on day 3 up to the highest test concentration of 300 mg/L.

## CONCLUSIONS

No statistical significant difference from the control was determined up to 300 mg/L on day 3. Therefore, the NOEC is determined to be 300 mg/L (nominal) and 696 mg/L (actual, based on CFU counts in the test solutions) corresponding to $6.5 \times 10^9$ CFU/L; and the LOEC is estimated to be > 300 mg/L (nominal) and > 696 mg/L (actual). The $E_rC_{50}$ and $E_yC_{50}$ could not be determined since the inhibition was not significant and below 50%.

**Toxic effects / Infectivity / Pathogenicity of plant protection product to algae**

| | |
|---|---|
| Test species | *Desmodesmus subspicatus* |
| Toxicity of plant protection product | Effects on algae: 72-hour (static): $EC_{50}$ > 696 mg/L |
| Infectivity / Pathogenicity | The duration of the test was too short to account for any infectivity and pathogenicity. |

**Comments and conclusion RMS:**

The test was performed according to OECD 201. The validity criteria according to this guideline were fulfilled.
Validity criteria OECD 201 (algae):

- Cell numbers, measured in the controls between 0 and 3 days increase by a factor of > 16 – met
- Coefficient of variation of average growth rate in replicate control cultures do not exceed 10% - met
- Mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) in control cultures do not exceed 35% - met

The study is considered acceptable. In a 72-h growth inhibition test, *Desmodesmus subspicatus* were exposed to CoStar WG at nominal concentrations of 0 (control) and 18.8, 37.5, 75.0, 150 and 300 mg/L. The average specific growth rate and the percentage of growth rates were determined on day 1, 2 and 3, and the yield was calculated.

No statistical inhibitory effects on growth rate and yield were observed on day 3 up to the highest test concentration of 300 mg/L.

No statistical significant difference from the control was determined up to 300 mg/L on day 3. Therefore, the NOEC is determined to be 300 mg/L (nominal) and 696 mg/L (actual); and the LOEC is estimated to be > 300 mg/L (nominal) and > 696 mg/L (actual). The $E_rC_{50}$ and $E_yC_{50}$ could not be determined since the inhibition was not significant and below 50%.

It can be concluded that under the condition of this test the product is not toxic to algae. The $LC_{50}$ > to $6.5 \times 10^9$ CFU/L, based on the CFU measured in the test solutions.

Endpoint:
Effects on algae: 72-hour (static): $EC_{50}$ > 696 mg/L corresponding to $6.5 \times 10^9$ CFU/L

## B.9.2.4 Effects on plants other than algae

*Bacillus thuringiensis subsp. kurstaki*, strain SA-12 are 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.

## B.9.2.5 Risk assessment for aquatic organisms

**Effects on aquatic organisms**

In this section, new studies are submitted assessing the effect of the product CoStar WG (Btk SA-12) on the aquatic organisms *Oncorhynchus mykiss*, *Daphnia magna* and *Desmodesmus subspicatus*.

Five studies are available which assess the effect of exposure of rainbow trout either to *Bacillus thuringiensis* subsp. *kurstaki* SA-11, Btk SA-12 or Btk EG2348 or the product CoStar WG. Two studies are available which assess the effect of exposure of daphnids either to *Bacillus thuringiensis* subsp. *kurstaki* SA-11 or the product CoStar WG and another two studies on the effect of exposure of single cell green algae either to Btk SA-12 or the product CoStar WG are presented here.

Due to the absence of adverse effects in these studies, it can be concluded that neither from the active ingredient Btk SA-12 nor from the co-formulants contained in CoStar WG any hazards to aquatic organisms are to be expected.

Results of the studies with aquatic organisms are summarised in **Table 9.2.5-1**.

**Table 9.2.5-1 Summary of the studies on effects for aquatic organisms**

| Test item | Test species | Endpoint* | Reference |
|---|---|---|---|
| **Fish** | | | |
| SAN 415 I (Btk SA-11) technical powder | *Oncorhynchus mykiss* | 30-day (semi static)<br>$LC_{50}$ > 41.5 mg /L[a)]<br>$LC_{50}$ > 1.0 × $10^9$ CFU/L | Vol. 3 MA, Section B.9, Point B.9.2.1 for Btk SA-11 |
| *Bacillus thuringiensis* EG2348 (Btk) | *Oncorhynchus mykiss* | 30-day (semi static)<br>$LC_{50}$ > 10 mg Btk/L [a)]<br>$LC_{50}$ > 1.0 × $10^9$ CFU/L<br>$LC_{50}$ > 5.3 mg Btk/L [b)] | Vol. 3 MA, Section B.9, Point B.9.2.1 for Btk EG2348 & EFSA Journal 2012;10(2):2540[9] |
| *Bacillus thuringiensis* EG2348 (Btk) | *Cyprinodon variegatus* | 32-day (semi static)<br>$LC_{50}$ > 100 mg Btk/L<br>$LC_{50}$ > 1.05 × $10^{10}$ CFU/L | Vol. 3 MA, Section B.9, Point B.9.2.1 for Btk EG2348 |
| Thuricide SC (SA-12) | *Danio rerio* | 30-day (semi static)<br>**$LC_{50}$ > 5.0 × $10^9$ CFU/L** | Vol. 3 MA, Section B.9, Point B.9.2.1 for Btk SA-12 |
| CoStar WG | *Oncorhynchus mykiss* | 96-hour (static limit)<br>$LC_{50}$ >51 mg Btk/L[c)] corresponding to 4.7 × $10^8$ CFU/L | Vol. 3 MP, Section B.9, Point B.9.2.1 for Btk SA-12 |
| **Aquatic invertebrates** | | | |
| SAN 415 I (Btk SA-11) technical powder | *Daphnia magna* | 48-hour (static)<br>$EC_{50}$ > 41.5 mg Btk/L<br>$EC_{50}$ > 1.0 × $10^9$ CFU/L[a)] | Vol. 3 MA, Section B.9, Point B.9.2.2 for Btk SA-11 |
| *Bacillus thuringiensis* EG2348 (Btk) | *Daphnia magna* | 21-day (semi static)<br>$EC_{50}$ > 41.5 mg /L[b)]<br>$EC_{50}$ > 8.4 × $10^8$ CFU/L | Vol. 3 MA, Section B.9, Point B.9.2.2 for Btk EG2348 & EFSA Journal 2012;10(2):2540[9] |
| Thuricide SC (SA-12) | *Daphnia magna* | 21-day (semi static)<br>**$EC_{50}$ > 1.0 × $10^9$ CFU/L** | Vol. 3 MA, Section B.9, Point B.9.2.2 for Btk SA-12 |
| CoStar WG[a)] | *Daphnia magna* | 48-hour (static)<br>$EC_{50}$ > 141 mg Btk/L[b) c)] corresponding to 1.3 × $10^9$ CFU/L | Vol. 3 MP, Section B.9, Point B.9.2.2 for Btk SA-12 |
| **Single cell algae** | | | |
| SAN 415 I (Btk SA-11) technical powder | *Selenastrum capricornutum* | 72-hour (static)<br>$EC_{50}$ > 42 mg/L<br>$EC_{50}$ > 1.0 × $10^9$ CFU/L[a)] | Vol. 3 MA, Section B.9, Point B.9.2.3 for Btk SA-11 |
| CoStar WG | *Desmodesmus subspicatus* | 72-hour (static)<br>**$EC_{50}$ > 696 mg/L[c)] corresponding to 6.5 × $10^9$ CFU/L** | Vol. 3 M9, Section B.9, Point B.9.2.3 for Btk SA-12 |

* Endpoints marked in **bold** are used for the risk assessment
[a)] Based on nominal concentrations
[b)] Actual concentration
[c)] This endpoint was not previously considered within the EU review process and is not EU-agreed.

[9] 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

**Risk Assessment**

Aquatic organisms may be exposed to Btk SA-12 entering surface waters via spray drift. The actual predicted environmental density of the population ($PED_{sw}$) of Btk SA-12 resulting from input via this route was initially estimated (please refer to Vol. 3 MP, Point B.8.1.2 for the calculation). The calculation was based on a worst case exposure scenario following 6 applications at 1.5 kg product/ha (1.275 kg a.s./ha) in pome fruits (orchards), assuming no degradation between the applications. This results in a $PED_{sw}$ of 276 µg/L or $1.57 \times 10^7$ CFU/L.

The risk of *Bacillus thuringiensis* subsp. *kurstaki* SA-12 to aquatic organisms was assessed from margin of safety (MOS; corresponding to TER) values according to the following equation:

$$MOS = \frac{EC_{50}[mg/L]}{PED_{sw}[mg/L]} \quad \text{or} \quad MOS = \frac{EC_{50}[CFU/L]}{PED_{sw}[CFU/L]}$$

Based on the available data the MOS values of fish, *Daphnia* and algae for Btk SA-12 was calculated (**Table 9.2.5-2**).

**Table 9.2.5-2 Margin of safety for aquatic organisms exposed to *B. thuringiensis***

| Use pattern | Test organism | $PED_{sw}$ [a)] | Endpoint | MOS |
|---|---|---|---|---|
| 6 × 1.275 kg a.s./ha in pome fruits | *Danio rerio* | $1.57 \times 10^7$ CFU/L | $5.0 \times 10^9$ CFU/L | 317 |
| | *Daphnia magna* | | $1.0 \times 10^9$ CFU/L | 63.5 |
| | *Desmodesmus subspicatus* | | $> 6.5 \times 10^9$ CFU/L | 414 |

[a)] Based on drift from accumulated applications, assuming no degradation between applications

Based on the submitted data on aquatic ecotoxicity and the intended use in fields and glasshouses, the calculated margin of safety values are high and it is anticipated that the potential risk posed from *Bacillus thuringiensis* subsp. *kurstaki* SA-12 to fish, *Daphnia* and algae is very low and acceptable.

| | |
|---|---|
| RMS evaluation | New studies are submitted assessing the effect of the product CoStar WG (Btk SA-12) on the aquatic organisms *Oncorhynchus mykiss*, *Daphnia magna* and *Desmodesmus subspicatus*.<br><br>Four studies are available which assess the effect of exposure of rainbow trout either to Btk SA-11, or Btk EG2348 or Btk SA-12 (CoStar WG). Three studies are available which assess the effect of exposure of daphnids either to Btk SA-11, Btk EG2348 or the product CoStar WG and another two studies on the effect of exposure of single cell green algae either to Btk SA-11 or CoStar WG are presented above. Due to the absence of adverse effects in these studies, it can be concluded that neither from the active ingredient Btk SA-12 nor from the co-formulants contained in CoStar WG any hazards to aquatic organisms are to be expected.<br><br>Based on the submitted data on aquatic eco-toxicity and the intended use in fields and glasshouses, the calculated margin of safety values are high and it is anticipated that the potential risk posed from *Bacillus thuringiensis* subsp. *kurstaki* SA-12 to fish, *Daphnia* and algae is very low and acceptable.<br><br>In conclusion the proposed use of CoStar WG does not pose an unacceptable risk to aquatic organism. |

## B.9.3 Effects on bees

### B.9.3.1 Toxicity, infectiveness and pathogenicity in bees

As CoStar WG was not the representative formulation for original approval of Btk SA-12 no data on the formulation have been submitted before.

**New data 2016**

It is referred to the information submitted for the active substance *Bacillus thuringiensis* subsp. *kurstaki* SA-12 in Vol.3 MA, Section B.98, Point B.9.3. The ingredients of the preparation CoStar WG, formulated as water dispersible granule, are inert and no hazards to the environment are expected (please refer to Vol. 4). Therefore, studies and information on the microbial pest control agent, *Bacillus thuringiensis* subsp. *kurstaki* SA-12, are considered applicable and relevant with regard to the evaluation of effects on bees of the formulated products.

In addition, a study is presented here assessing the effects of Delfin WG on honeybees. Delfin WG contains Btk SA-11 which is closely related and very similar to Btk SA-12. In addition, Delfin WG and CoStar WG are identical with regard to the product composition. The study on Delfin WG is therefore considered fully applicable to assess possible effects of CoStar WG on honeybees.

| | |
|---|---|
| Report: | KMP 9.3.1/01 - Vergé, E. (2016) |
| Title: | Delfin WG - Acute oral and contact toxicity to the Honey bee, *Apis mellifera* L. under laboratory conditions |
| Document No: | S15-05620 |
| Guidelines: | OECD 213/214 (1998) and OPPTS 885.4380 (1996) |
| GLP | yes |
| Validity | yes |

**Executive Summary**

The product Delfin WG was tested for its acute oral contact and toxicity on honey bees in a limit test design. The duration of the oral and contact toxicity test was 19 and 15 days, respectively. One control, one dose of the test item and 4 doses of the reference item were tested with 5 replicates of 10 bees per treatment group. Assessment of mortality was performed 4, 24, 48, 72 and 96 hours after test start. Thereafter mortality was recorded every 24 hours up to 19 days for oral toxicity and 15 days for contact toxicity. The reference item group observation period was 48 hours.

In the control group of the oral toxicity test, 2.0% mortality was recorded after 96 hours. A mortality of 20.0% was recorded 19 days after application. For the test item dose of 100 µg product/bee (actual uptake: 82 µg product/bee), a mortality of 24% (corrected mortality: 5.0%) was observed 19 days after start of feeding. This was not statistically significantly different compared to the control; the NOEC is therefore greater than 82 µg product/bee. One affected bee was recorded 14 and 19 days after test start, respectively.

In the control group of the contact toxicity test, 2.0% mortality was recorded after 96 hours. A mortality of 20.0% was recorded 15 days after application. For the test item dose of 100 µg product/bee, a mortality of 8.0% (corrected mortality: -15.0%) was recorded 15 days after start of the test. This was not statistically significantly different compared to the control; the NOEC is therefore greater than 100 µg product/bee. No behavioural abnormalities were recorded during the 15 days test period.

At the end of the 19 day test period the oral $LD_{50}$ was > 82 µg/bee, equivalent to 4.2 x $10^6$ cfu/bee and at the end of the 15 day test period the contact $LD_{50}$ was > 100µg/bee equivalent to 5.1 x $10^6$ cfu/bee.

**MATERIALS AND METHODS**

**Test Item**

| | |
|---|---|
| Designation | Delfin WG |
| Active ingredient | *Bacillus thuringiensis kurstaki* |

| | |
|---|---|
| Characteristics | Brown solid |
| Batch no. | 2501595 |
| Expiration date | 07 October 2016 |
| Purity | 5.1 x $10^{10}$ cfu/g |

**Test System**

| | |
|---|---|
| Species | Honey bee (*Apis mellifera* L.) |
| Age | 3-4 day old female bees |
| Origin | Queen-right, healthy colony from a breeding line of a beekeeper in Montroy, Spain |
| Food | 50% (w/v) aqueous sucrose solution *ad libitum* |

**Test Conditions**

| | |
|---|---|
| Test duration | 19 days (oral toxicity) and 15 days (contact toxicity) |
| Temperature | 30.1 - 34.4°C |
| Rel. Humidity | 42.4 - 68.4% |
| Illumination | darkness |

**Study Design and Methods**

| | |
|---|---|
| In-life dates | November 05, 2015 to December 13, 2015 |
| Experimental treatment | Deionised water was used as solvent for the test item. Further dilutions of the stock solution(s) were prepared using 50% (w/v) aqueous sucrose solution (oral toxicity test) and deionised water (contact toxicity test).<br>Contact test:<br>A hand operated micro-applicator (Burkhard Ltd.) was used for application. The application amount was 2 µL per bee. A 2-µL droplet was chosen instead of 1-µL droplet since a higher volume ensures a more reliable dispersion of the application solutions. After having been anaesthetised with CO2, the droplet of the application solution was applied individually to the dorsal side of the thorax of the bee.<br>Oral test:<br>The application volume was 200µL per replicate. The bees in one replicate share the application solution and thus receive similar doses. The bees were starved for approx. 2 hours prior to application start. Each unit was provided with the application solution for up to 6 hours to ensure sufficient uptake. The feeders were then removed and the bees were provided *ad libitum* with untreated 50% (w/v) aqueous sucrose solution<br>Dosage of the Test Item:<br>Contact test: 100 µg product/bee<br>Oral test: 100 µg product/bee (target dose) / 82 µg product/bee (actual dose)<br>Dosage of the reference item:<br>4 doses were tested:<br>Contact test: 0.08, 0.12, 0.18, 0.27 µg dimethoate/bee<br>Oral test: 0.06, 0.09, 0.14, 0.21 µg/bee (target dose) / 0.03, 0.06, 0.07, 0.16 µg/bee (actual dose) |
| Replicates | 5 |
| Bees/replicate | 10 |
| Observations | Mortality and behavioural abnormalities: after 4, 24, 48, 72 and 96 hours after test start. Thereafter mortality was recorded every 24 hours up to 19 days for oral toxicity and 15 days for contact toxicity |

**RESULTS AND DISCUSSION**

In the control group of the oral toxicity test, 2.0% mortality was recorded after 96 hours. A mortality of 20.0% was recorded 19 days after application. For the test item dose of 100 µg product/bee (actual uptake: 82 µg product/bee), a mortality of 24% (corrected mortality: 5.0%) was observed 19 days after start of feeding. This was not statistically significantly different compared to the control; the NOEC is therefore greater than 82 µg product/bee. One affected bee was recorded 14 and 19 days after test start, respectively.

In the control group of the contact toxicity test, 2.0% mortality was recorded after 96 hours. A mortality of 20.0% was recorded 15 days after application. For the test item dose of 100 µg product/bee, a mortality of 8.0% (corrected mortality: -15.0%) was recorded 15 days after start of the test. This was not statistically significantly different compared to the control; the NOEC is therefore greater than 100 µg product/bee. No behavioural abnormalities were recorded during the 15 days test period.

The counted numbers of colony forming units (cfu) of *Bacillus thuringiensis kurstaki* in the test item were in the range from 77 to 154% of the nominal number of colonies. On average 102% of the nominal number of colonies of 5.1 x $10^{10}$ cfu/g (based on the content given in the CoA) was found. This corresponds to an actual content of 5.2 x $10^{10}$ cfu/g.

**CONCLUSIONS**

The oral and contact 4, 24, 48, 72 and 96 hour $LD_{50}$ for Delfin WG is > 82µg/bee, equivalent to 4.2 x $10^6$ cfu/bee and > 100µg/bee equivalent to 5.1 x $10^6$ cfu/bee, respectively.

At the end of the 19 day test period the oral $LD_{50}$ was > 82µg/bee, equivalent to 4.2 x $10^6$ cfu/bee and at the end of the 15 day test period the contact $LD_{50}$ was > 100µg/bee equivalent to 5.1 x $10^6$ cfu/bee.

**Toxic effects / Infectivity / Pathogenicity of plant protection product to bees**

| | |
|---|---|
| Test species | Honey bee (*Apis mellifera*) |
| Toxicity of plant protection product | Oral toxicity (15 d):<br>$LD_{50}$ > 82 µg product/bee or > 4.2 × $10^6$ CFU/bee<br>Contact toxicity (19 d):<br>$LD_{50}$ > 100 µg product/bee or > 5.1 × $10^6$ CFU/bee |
| Infectivity / Pathogenicity | Pathogenicity and infectivity not tested |

**Comments and conclusion RMS:**

The product Delfin WG was tested for its acute oral and contact toxicity on honey bees in a limit test design. The duration of the oral and contact toxicity test was 19 and 15 days, respectively. The study is considered acceptable even though control and treated bees should be observed for at least 30 days after dosing according to OPPTS 885.4380 guideline. It is noted that the 30-day exposure period which is required according to OPPTS is rather unrealistic to achieve. Usually mortality in control groups strongly increases after a certain time period exceeding the 20% which is the validity criterion for the studies.

In the control group of the oral toxicity test, 2.0% mortality was recorded after 96 hours. A mortality of 20.0% was recorded 19 days after application. For the test item dose of 100 µg product/bee (actual uptake: 82 µg product/bee), a mortality of 24% (corrected mortality: 5.0%) was observed 19 days after start of feeding. This was not statistically significantly different compared to the control; the NOEC is therefore greater than 82 µg product/bee. One affected bee was recorded 14 and 19 days after test start, respectively.

In the control group of the contact toxicity test, 2.0% mortality was recorded after 96 hours. A mortality of 20.0% was recorded 15 days after application. For the test item dose of 100 µg product/bee, a mortality of 8.0% (corrected mortality: -15.0%) was recorded 15 days after start of the test. This was not statistically significantly different compared to the control; the NOEC is therefore greater than 100 µg product/bee. No behavioural abnormalities were recorded during the 15 days test period.

At the end of the 19 day test period the oral $LD_{50}$ was > 82 µg/bee, equivalent to 4.2 x $10^6$ cfu/bee and at the end of the 15 day test period the contact $LD_{50}$ was > 100 µg/bee equivalent to 5.1 x $10^6$ cfu/bee.

| | |
|---|---|
| RMS evaluation | The ingredients of the preparation CoStar WG, formulated as water dispersible granule, are inert and no hazards to the environment are expected (please refer to Vol. 4). Therefore, studies and information on the microbial pest control agent, *Bacillus thuringiensis* subsp. *kurstaki* SA-12, are considered applicable and relevant with regard to the evaluation of effects on bees of the formulated products. However the study results of two studies assessing the side effects of oral and contact exposure of the active substance *Bacillus thuringiensis* subsp. *kurstaki* SA-12 can only be used as indicative in the risk assessment. Please refer to the information submitted in Vol.3 MA, Section B.98, Point B.9.3.<br><br>Since, the product Delfin WG (which is the product in the study assessed above) only contains the micro-organisms Btk SA-11 and an inert co-formulant studies performed on the product is considered applicable also to cover data for the active substance. Btk SA-11 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[10] 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.<br><br>The study on Delfin WG is therefore considered fully applicable to assess possible effects of CoStar WG on honeybees and can be used in the risk assessment. |
| Endpoint:<br>Effects on bees | Endpoint (Delfin WG, containing Btk SA-11)<br>Oral toxicity (15 d):<br>$LD_{50}$ > **82 µg product/bee** or **> 4.2 × $10^6$ CFU/bee**<br>Contact toxicity (19 d):<br>$LD_{50}$ > **100 µg product/bee** or **> 5.1 × $10^6$ CFU/bee** |

## B.9.3.2 Risk assessment for bees

**Effects on bees**

Data on another Btk strain, EG2348, have already been evaluated as part of the EU review for the inclusion of Btk SA-12 into Annex I (now list of approved active substances according to (EC) No 1107/2009). Furthermore, two studies are submitted assessing the acute oral and acute contact toxicity of Thuricide SC (liquid formulation of Btk SA-12) to honeybees. Additionally, a new study is submitted assessing the effect of the product Delfin WG on bees. This study included a prolonged observation time in order to assess potential pathogenic effects after oral and contact exposure. For details see study KMP 9.3.1/01 above. Delfin WG contains Btk SA-11 which is closely related and very similar to Btk SA-12. In addition, Delfin WG and CoStar WG are identical with regard to the product composition. The study on Delfin WG is therefore considered fully applicable to assess possible effects of CoStar WG on honeybees.

The new and EU agreed endpoints are summarised in **Table 9.3.2-1**.

Bees may be exposed to Btk SA-12 by direct over-spray, by contact with residues on plants while foraging, or by consumption of contaminated, pollen, nectar or water. The intended use of CoStar WG is by spray application in pome fruits and ornamentals. Therefore, exposure cannot be excluded.

For the risk assessment the maximum single application rate of $1.14 \times 10^{14}$ CFU/ha (equivalent to 1.7 kg a.s./ha and 2.0 kg product/ha) in ornamentals will be considered. For a detailed summary of the proposed uses of CoStar WG, please refer to Critical GAP provided in introduction.

[10] 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

**Table 9.3.2-1 Summary of the studies on effects to bees**

| Test substance | Test species | Endpoint* | Reference |
|---|---|---|---|
| *Bacillus thuringiensis* EG2348 (Btk) | *Apis mellifera* | 5-d $LD_{50}$ > 25 µg a.s./bee | EFSA Journal 2012;10(2):2540[11] |
| Thuricide SC (SA-12) | *Apis mellifera* | Oral toxicity (4 d): $LD_{50} > 1 \times 10^9$ CFU/L | Vol. 3 MA, Section B.9, Point B.9.3 for Btk SA-12 |
| Thuricide SC (SA-12) | *Apis mellifera* | Contact toxicity (5 d): $LD_{50} > 1 \times 10^9$ CFU/L | Vol. 3 MA, Section B.9, Point B.9.3 for Btk SA-12 |
| Delfin WG[1)] | *Apis mellifera* | Oral toxicity (15 d): $LD_{50}$ > **82 µg product/bee** or **> 4.2 × $10^6$ CFU/bee** Contact toxicity (19 d): $LD_{50}$ > **100 µg product/bee** or **> 5.1 × $10^6$ CFU/bee** | Vol. 3 MP, Section B.9, Point B.9.3 for Btk SA-11 |

* Endpoints marked in **bold** are used for the risk assessment
[1)] Delfin WG contains Btk SA-11, which is closely related and very similar to Btk SA-12

**Risk assessment for bees**

The calculation of HQ values as used for chemicals (application rate/$LD_{50}$) is generally regarded as less feasible for risk assessments with mBCAs because dose-response relationships are rarely observed in cases of pathogenic effects (OECD 2012[12]).

According to the Commission Regulation (EU) No 546/2001, Part II, Uniform principles for evaluation and authorisation of plant protection products containing micro-organisms[13], Part B, article 2.8.3.1, a micro-organism may give rise to risks because of its potential to infect and multiply in bees. Whether or not identified risks could be changed due to the formulation of the plant protection product shall be assessed, taking into account the following information on the micro-organism:

(a) its mode of action,

(b) other biological properties,

(c) studies on toxicity, pathogenicity and infectivity to honeybees and other arthropods.

Also in the Commission Regulation (EU) No 546/2001, Part II, Uniform principles for evaluation and authorisation of plant protection products containing micro-organisms[12], Part C, article 2.8.3., where there is a possibility of bees being exposed, no authorisation shall be granted if:

(a) the micro-organism is pathogenic to bees,

(b) in case of toxic effects due to components in the plant protection product such as relevant metabolites/toxins, the hazard quotients for oral or contact exposure of honeybees are greater than 50, unless it is clearly established through an appropriate risk assessment that under field conditions there are no unacceptable effects on honeybee larvae, honeybee behaviour, or colony survival and development after use of the plant protection product according to the proposed conditions of use.

In order to address the requirement for an exposure assessment for bees following GAP directed use of CoStar WG an approach is used comparing the worst case field exposure (concentration/density in spraying solution) with the concentrations of Delfin WG/densities of Btk SA-11 used in the presented bee study above.

Taking into consideration the fact that products containing *B. thuringiensis* have a different mode of action compared to chemical pesticides, the standard test designs have been adapted in terms of test duration. The endpoints

[11] 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
[12] OECD Guidance to the Environmental Safety Evaluation of Microbial Biocontrol Agents, Series on Pesticides No. 67, ENV/JM/MONO(2012)1
[13] Commission Regulation (EU) No 546/2011: Uniform Principles for Evaluation and Authorisation of Plant Protection Products, as provided for in Article 29(6) of Regulation (EC) No 1107/2009

obtained after this prolonged observation time are set into relation with the maximum concentration of Delfin WG in the spraying liquid. In the studies with Delfin WG 100 µg/bee were applied in 20 µL/bee in the oral test and in 2 µL/bee in the contact test. Honey bees exposed to test item suspensions with similar or even higher density than the intended use did not show any symptoms of toxicity or pathogenicity after an observation time of at least 15 days.

The risk of *Bacillus thuringiensis* subsp. *kurstaki* SA-12 to honeybees was assessed from margin of safety (MOS; corresponding to TER) values according to the following equation:

$$\mathrm{MOS} = \frac{\text{Test concentration}\,[\mathrm{CFU/L}]}{\text{max. field concentration}\,[\mathrm{CFU/L}]}$$

Based on the available data the MOS values of honeybee for oral and contact exposure for Btk SA-12 was calculated (**Table 9.3.2-2**).

**Table 9.3.2-2 Exposure assessment for bees**

| Crop scenario | Single AR [a] | Minimum Water | Maximum concentration | Exposure | Concentration in test solution | MOS Test / field |
|---|---|---|---|---|---|---|
| Ornamentals | $1.14 \times 10^{14}$ CFU/ha | 500 L/ha | $2.28 \times 10^{11}$ CFU/L | oral | $2.55 \times 10^{11}$ CFU/L | 1.12 |
| | | | | contact | $2.55 \times 10^{12}$ CFU/L | 11.2 |

MOS = Margin of safety
[a] Maximum single application rate

Due to the absence of symptoms of toxicity or pathogenicity during the test, an acceptable acute risk by contact and oral exposure can be concluded for honey bees for the GAP use envisaged.

| | |
|---|---|
| RMS evaluation | According to the EFSA peer review of the risk assessment of the five Btk strains[1] the extrapolation between different *Bacillus thuringiensis* subsp. *kurstaki* strains can be considered acceptable for non-target organisms, except for daphnids and non-target arthropods.<br><br>Based on the mode of action to their insect host, *Bacillus thuringiensis* subsp. *kurstaki* SA-12 and the biological properties of the natural occurring organism, no risk to bees are expected. Btk SA-12, like other approved Btk strains, 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. In the available studies no toxicity, infectivity and pathogenicity is reported at the highest tested dose level.<br><br>Bees may be exposed to Btk SA-12 by direct over-spray, by contact with spray deposits on plants while foraging, or by consumption of contaminated pollen, nectar or water. The intended use of CoStar WG is by spray application in pome fruits and ornamentals. Therefore, exposure to bees cannot be excluded.<br><br>In order to address the requirement for an exposure assessment for bees following GAP directed use of CoStar WG an approach is used comparing the worst case field exposure with the concentrations of CoStar WG/densities of Btk SA-12 used in the new bee study. Due to the absence of symptoms of toxicity or pathogenicity during the test, an acceptable acute risk by contact and oral exposure can be concluded for honey bees for the GAP use envisaged.<br><br>In conclusion the proposed use of CoStar WG does not pose an unacceptable risk to bees. |

## B.9.4 Effects on arthropods other than bees

### B.9.4.1 Toxicity, infectiveness and pathogenicity in arthropods other than bees

As CoStar WG was not the representative formulation for original approval of Btk SA-12 no data on the formulation have been submitted before.

**New data 2016**

It is referred to the information submitted for the active substance *Bacillus thuringiensis* subsp. *kurstaki* SA-12 in Vol 3. MA, Section B.9, Point B.9.4.

In addition, two studies are presented here assessing the effects of CoStar WG on non-target arthropods other than bees - *Typhlodromus pyri* and *Aphidius rhopalosiphi.*

| | |
|---|---|
| Report: | KMP 10.4/01 - Walter, C. (2014) |
| Title: | CoStar: Toxicity to the predatory mite *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae) under laboratory conditions |
| Document No: | S13-04875 |
| Guidelines: | IOBC (Blümel *et al*., 2000); ESCORT I Guidance Document (Barrett *et al*., 1994) and ESCORT II Guidance Document (Candolfi *et al*., 2001) |
| GLP | yes |
| Validity | yes |

**Executive Summary**

The purpose of this study was to determine effects of CoStar WG on mortality and reproduction of *Typhlodromus pyri* under worst-case conditions. For assessment of mortality of *Typhlodromus pyri*, protonymphs (age ≤ 24 hours) were exposed to glass plates treated with CoStar WG. The test item was applied with a laboratory track sprayer at the rate of 12000 g product/ha in a spray volume of 200 L/ha. The mortality and escaping rated of the juvenile mites was assessed up to the adult stage, on day 3 and day 7 of exposure. On day 7, the sex ratio was determined. During the following 7-day exposure period, the reproduction per female was evaluated on day 10, 12 and 14 after start of exposure. Reproduction was assessed in the test item group where the corrected mortality was ≤ 50%.

The mean 7-day mortality (defined as the number of dead and escaped mites) was 31% in the test item group compared to 10.0% mortality in the control group. The corrected mortality was calculated as 23.3%.

The mean 7-day escaping rate (defined as the mites, which are stuck in the glue, found in the water supply or missing mites) was 21.0% in the test item group compared to 9.0% escaping rate in the control group. The corrected escaping rate was calculated as 13.2%.

The mean number of eggs per female produced during the reproduction period until day 14 was 6.2 in the test item group compared to 7.1 eggs per female in the control group. The reduction in reproduction was calculated as 12.7%.

With respect to the results it can be concluded that CoStar WG caused a statistically significant effect on mortality of *Typhlodromus pyri* at the rate of 12000 g product/ha. However, the effect was clearly below the trigger value of 50%. The reproduction of *T. pyri* was not significantly reduced compared to the control at the rate of 12000 g product/ha.

**MATERIALS AND METHODS**

**Test Item**

| | |
|---|---|
| Designation | CoStar WG |
| Active ingredient | *Bacillus thuringiensis kurstaki* SA-12 |
| Characteristics | Brown solid |

| | |
|---|---|
| Batch no. | SR132041 |
| Expiration date | 17.10.2014 |
| Purity | 90000 IU/mg |

**Test System**

| | |
|---|---|
| Species | *Typhlodromus pyri* Scheuten |
| Age | protonymphs, not older than 24 hours |
| Origin | Healthy rearing stock at the testing facility |
| Number | 20 per replicate (5 replicates per treatment group) |
| Food | Pollen of bean (*Vicia faba*) and birch (*Betula pendula*) |

**Test Conditions**

| | |
|---|---|
| Temperature | 24.5 - 26.0°C |
| Photoperiod | 16 h light : 8 h dark |
| Light intensity | 2200 – 3300 Lux |
| Rel. Humidity | 63.5 - 79.6% |

**Study Design and Methods**

| | |
|---|---|
| In-life dates | 08.11.2013 to 22.11.2013 |
| Experimental treatment | The test item was applied to glass plates at a spray volume of 200 L/ha in a single application. Each test unit consisted of two glass cover slides which were placed together with their longitudinal sides. They were fixed together by means of two glass bars which were glued on them on the upper surface. In order to prevent the mites from escaping, a non-drying glue gel was applied on the centre of the glass cover slides. The glue barrier was formed as a square arena which resulted in an exposure area of approx. 10 - 13 $cm^2$. The glue barrier was set up before application.<br>After the application, 20 individuals (5 replicates per treatment group) were introduced with a fine brush after drying of the spray layer, within 47 min after application. |
| Observations | Mortality:<br>The number of living, dead and escaped mites was assessed at day 3 and at day 7 after test initiation.<br>Reproduction<br>On day 7 the sex of the test organisms was determined. Compensation of sex ratio was not necessary as it was at least 1 male to 5 females for each treatment group at the start of the reproduction test. The number of offspring per female was determined by counting the number of females and eggs/larvae on day 10, 12 and 14 of exposure. Eggs laid until day 7 inclusive were removed from the test arena. At each assessment, males and females were counted and the number of eggs and juveniles were determined. Dead animals, eggs and juvenile mites were removed after counting. |

**RESULTS AND DISCUSSION**

The mean 7-day mortality (defined as the number of dead and escaped mites) was 31% in the test item group compared to 10.0% mortality in the control group. The corrected mortality was calculated as 23.3%.

The mean 7-day escaping rate (defined as the mites, which are stuck in the glue, found in the water supply or missing mites) was 21.0% in the test item group compared to 9.0% escaping rate in the control group. The corrected escaping rate was calculated as 13.2%.

The mean number of eggs per female produced during the reproduction period until day 14 was 6.2 in the test item group compared to 7.1 eggs per female in the control group. The reduction in reproduction was calculated as 12.7%.

**CONCLUSIONS**

With respect to the results it can be concluded that CoStar caused a statistically significant effect on mortality of *Typhlodromus pyri* at the rate of 12000 g product/ha. However, the effect was clearly below the trigger value of 50%. The reproduction of *T. pyri* was not significantly reduced compared to the control at the rate of 12000 g product/ha corresponding to $1.1 \times 10^{12}$ IU/ha.

**Toxic effects / Infectivity / Pathogenicity of plant protection product to arthropods other than bees**

| | |
|---|---|
| Test species | Predatory mite, *Typhlodromus pyri* |
| Toxicity of plant protection product | $LR_{50}$ > 12 kg CoStar WG/ha corresponding to $1.1 \times 10^{12}$ IU/ha |
| Infectivity / Pathogenicity | Pathogenicity and infectivity not tested |

**Comments and conclusion RMS:**

The test was conducted according to the IOBC guidelines. The validity criteria as per guideline were fulfilled. Infectivity and pathogenicity were not investigated. The study is considered acceptable to cover current requirements.

In limited laboratory tests CoStar WG was tested for acute toxicity to the predatory mite *Typhlodromus pyri*.

CoStar WG caused a statistically significant effect on mortality of *T. pyri* at an application rate of 12 kg/ha with a calculated mortality of 23%. However, the effect was clearly below the trigger value of 50%. The reproduction of *T. pyri* was not significantly reduced in the test item group and the reduction in reproduction was calculated as 12.7%.

Consequently, the endpoint was $LR_{50}$ > 12 kg CoStar WG/ha corresponding to $1.1 \times 10^{12}$ IU/ha.

| | |
|---|---|
| Report: | KMP 10.4/02 - Walter, C. (2016) |
| Title: | CoStar: Toxicity to the aphid parasitoid *Aphidius rhopalosiphi* (DeStefani Perez) (Hymenoptera, Braconidae) under laboratory conditions |
| Document No: | S15-01102 |
| Guidelines: | IOBC (Mead-Briggs *et al*., 2000); ESCORT I Guidance Document (Barrett *et al*., 1994) and ESCORT II Guidance Document (Candolfi *et al*., 2001) |
| GLP | yes |
| Validity | yes |

**Executive Summary**

The purpose of this study was to determine effects of CoStar WG on mortality and reproduction of *Aphidius rhopalosiphi* under worst-case conditions. For assessment of mortality of *Aphidius rhopalosiphi*, protonymphs (age ≤ 24 hours) were exposed to glass plates treated with CoStar WG. The test item was applied with a laboratory track sprayer at the rate of 12000 g product/ha in a spray volume of 200 L/ha. The mortality was assessed 2 h, 24 and 48 hours. After the 48-hour exposure period, reproduction (parasitisation rate) was evaluated by transferring 17 female wasps from each test item group and the control group to individual test units containing barley seedlings infested with aphids (*Rhopalosiphum padi*). Following a 24-h parasitation period the wasps were removed and the plants and aphids (parasitised) were held for another period of 10 days after which the number of parasitised aphids (aphid mummies) was determined.

After 48-h of exposure on the glass plates the mean mortality was 2.5% in the test item group compared to 0.0% mortality in the control group. The corrected mortality was calculated as 2.5%. No abnormal behaviour of the wasps was observed on the test item group compared to the control.

The reproduction (parasitisation rate) was assessed in the test item group and the control group. The reproduction in the test item group was slightly better (22.3 mummies per female) compared to the control group (21.1 mummies per female).

With respect to the results it can be concluded that CoStar WG caused no adverse effect on mortality and reproduction (parasitisation rate) of *Aphidius rhopalosiphi* at 12000 g product/ha, when compared to the control.

## MATERIALS AND METHODS

### Test Item

| | |
|---|---|
| Designation | CoStar WG |
| Active ingredient | *Bacillus thuringiensis kurstaki* SA-12 |
| Characteristics | Brown granular |
| Batch no. | 0115-85 |
| Expiration date | 04.03.2016 |
| Purity | $\geq 8.5 \times 10^{12}$ cfu/kg |

### Test System

| | |
|---|---|
| Species | *Aphidius rhopalosiphi* De Stefani Perez |
| Age | Adult wasps, not older than 48 hours after hatching |
| Origin | Katz Biotech AG, An der Birkenpfuhlheide 10, D-15837 Baruth, Germany |
| Number | 10 adults per replicate (4 replicates per treatment group) |
| Food | Honey water gelatine solution (100 g honey, 50 g aqua dest., 1.5 g gelatine) and a aqueous sucrose solution (20% saccharose) *ad libitum* |

### Test Conditions

| | |
|---|---|
| Temperature | 19.9 - 21.6°C |
| Photoperiod | 16 h light : 8 h dark |
| Light intensity | ~1900 Lux (during exposure)<br>800 - 2500 Lux (during parasitisation)<br>8300 - 12000 Lux (during aphid development of mummies) |
| Rel. Humidity | 59.5 - 74.9% |

### Study Design and Methods

| | |
|---|---|
| In-life dates | 30.03.2015 to 20.04.2015 |
| Experimental treatment | Exposure:<br>The test item was applied to glass plates at a spray volume of 200 L/ha in a single application.<br>After the application, 10 individuals (4 replicates per treatment group) were introduced after drying of the spray layer, the sex ratio (male : female) was 2:8 in all treatment groups.<br>Reproduction:<br>A Plexiglas tube was place upon a pot containing aphid infested barley seedlings. The top of the tube was covered with gauze. |
| Observations | Mortality:<br>The condition of the test organisms was observed approx. 2 h, 24 h and 48 hours after introduction.<br>Reproduction<br>Surviving females were removed from the exposure units and transferred individually to the reproduction units at the end of the 48-hour exposure |

period. After an approx. 24 hour parasitisation period the females were removed from the reproduction units and their condition (alive, dead or not recovered) was recorded. The number of parasitised aphids was counted in each replicate 10 days after the end of the parasitisation period (13 days after start of exposure).

**RESULTS AND DISCUSSION**

After 48-h of exposure on the glass plates the mean mortality was 2.5% in the test item group compared to 0.0% mortality in the control group. The corrected mortality was calculated as 2.5%. No abnormal behaviour of the wasps was observed on the test item group compared to the control.

The reproduction (parasitisation rate) was assessed in the test item group and the control group. The reproduction in the test item group was slightly better (22.3 mummies per female) compared to the control group (21.1 mummies per female).

**CONCLUSIONS**

With respect to the results it can be concluded that CoStar WG caused no adverse effect on mortality and reproduction (parasitisation rate) of *Aphidius rhopalosiphi* at 12000 g product/ha (corresponding to $1.1 \times 10^{12}$ IU/ha), when compared to the control.

**Toxic effects / Infectivity / Pathogenicity of plant protection product to arthropods other than bees**

| | |
|---|---|
| Test species | Aphid parasitoid hymenopteras, *Aphidius rhopalosiphi* |
| Toxicity of plant protection product | $LR_{50}$ > 12 kg CoStar WG/ha corresponding to $1.1 \times 10^{12}$ IU/ha |
| Infectivity / Pathogenicity | Pathogenicity and infectivity not tested |

**Comments and conclusion RMS:**

The test was conducted according to the IOBC guidelines. The validity criteria as per guideline were fulfilled. Infectivity and pathogenicity were not investigated. The study is considered acceptable to cover current requirements.

In limited laboratory tests CoStar WG was tested for acute toxicity to the aphid parasitoid hymenopteras *Aphidius rhopalosiphi*.

CoStar WG caused no adverse effect on mortality and reproduction of *A. rhopalosiphi* at an application rate of 12 kg/ha, in terms of potency equivalent to 1.1 x $10^{12}$ IU/ha. Consequently the endpoint was $LR_{50}$ (lethal ratio causing 50% mortality) > 12 kg CoStar/ha corresponding to $1.1 \times 10^{12}$ IU/ha).

## B.9.4.2 Risk assessment for arthropods other than bees

**Effects on arthropods other than bees**

The acute toxicity and effect on reproduction of CoStar WG to the aphid parasitoid *Aphidius rhopalosiphi* (Hymenoptera, Braconidae) and the predatory mite *Typhlodromus pyri* (Acari, Phytoseiidae; both standard indicator species) was determined in a laboratory limit test studies. Statistically significant effects on survival (23.3%) were observed in worst case laboratory tests with *T. pyri* at the tested rate of 12.0 kg CoStar WG/ha. No significant effects have been observed when *A. rhopalosiphi* was exposed to the same rate. Effects on reproduction did not occur in either species. These studies are summarised above.

The toxicity of the formulation to non-target arthropods has been investigated by carrying out Tier I test on *Aphidius rhopalosiphi* and *Typhlodromus pyri*. Both studies confirmed the absence of toxicity of the test item to non-target arthropods. An overview on the obtained data is provided in **Table 9.4.2-1**.

**Table 9.4.2-1 Summary of the studies on effects to non-target arthropods**

| Test substance | Species | Exposed life stage | Study type | Endpoint | Reference |
|---|---|---|---|---|---|
| CoStar WG | *Aphidius rhopalosiphi* | Adult | Acute laboratory (glass plate) | $EC_{50}$ > 12 kg product/ha corresponding to 1.1 × $10^{12}$ IU/ha | Doc M-MP, Sec. 10, KMP 10.4/02 |
| | *Typhlodromus pyri* | Protonymphs | Acute laboratory (glass plate) | $EC_{50}$ > 12 kg product/ha corresponding to 1.1 × $10^{12}$ IU/ha | Doc M-MP, Sec. 10, KMP 10.4/01 |

**Risk assessment for arthropods other than bees**

The calculation of HQ values as used for chemicals (application rate/$LD_{50}$) is generally regarded as less feasible for risk assessments with mBCAs because dose-response relationships are rarely observed in cases of pathogenic effects (OECD 2012[14]).

The risk of *Bacillus thuringiensis* subsp. *kurstaki* SA-12 to non-target arthropods other than bees was assessed from margin of safety (MOS; corresponding to TER) values according to the following equation:

$$\mathrm{MOS} = \frac{\mathrm{EC}_{50}\ [\text{g product/ha}]}{\text{applicatio n rate}\ [\text{g product/ha}]}$$

The resulting values for the single application rates in pome fruits and in ornamentals and for the maximum application rate in pome fruits and ornamentals are presented in Table 9.4.2-2 and in Table 9.4.2-3, respectively.

To include a parameter which is relevant for the assessment of a microorganism, International Units are used. International Units as a parameter for the biopotency directly reflect the insecticidal activity of a Bt strain or product and are as such considered most relevant for the risk assessment for non-target arthropods.

**Table 9.4.2-2 Exposure Assessment for the single application rate of 1.5 kg CoStar WG/ha in pome fruits and 2 kg CoStar WG in ornamentals**

| Crop | $EC_{50}$ [g product/ha] | Single application rate [g product/ha] | MOS |
|---|---|---|---|
| Pome fruits | > 12000 or > 1.1 × $10^{12}$ IU/ha | 1500<br>1.4× $10^{11}$ IU/ha | 8.0<br>7.9 |
| Ornamentals | | 2000<br>1.8× $10^{11}$ IU/ha | 6.0<br>6.1 |

MOS = Margin of safety

[14] OECD Guidance to the Environmental Safety Evaluation of Microbial Biocontrol Agents, Series on Pesticides No. 67, ENV/JM/MONO(2012)1

**Table 9.4.2-3 Exposure Assessment for the accumulated maximum application rate of 9 kg CoStar WG/ha in pome fruits and 12 kg CoStar WG in ornamentals**

| Crop | $EC_{50}$ [g product/ha] | Accumulated maximum application rate [g product/ha] | MOS |
|---|---|---|---|
| Pome fruits | > 12000 or > $1.1 \times 10^{12}$ IU/ha | 9000 or $8.1 \times 10^{11}$ IU/ha | 1.3<br>1.4 |
| Ornamentals | | 12000 or $1.1 \times 10^{12}$ IU/ha | 1.0<br>1.0 |

MOS = Margin of safety

When based on g formulated product/ha or International units, an acceptable margin of safety is derived for the exposure to non-target arthropods after the use of CoStar WG according to GAP based on multiple applications. The application rate is summed in this calculation. It is very unlikely that the same population of non-target arthropods is exposed to each application. Furthermore, it is extremely worst-case to assume a cumulative application rate as the both active microorganism and the product will not be stable on the crop due to environmental conditions. It can be therefore concluded that the risk for NTA following GAP directed use of CoStar WG is acceptable.

According to the Commission Regulation (EU) No 546/2001, Part II, Uniform principles for evaluation and authorisation of plant protection products containing micro-organisms[15], Part B, article 2.8.4.1, a micro-organism may give rise to risks because of its potential to infect and multiply in arthropods other than bees. Whether or not identified risks could be changed due to the formulation of the plant protection product shall be assessed, taking into account the following information on the micro-organism:

(a) its mode of action,

(b) other biological properties,

(c) studies on toxicity, pathogenicity and infectivity to honeybees and other arthropods.

And in article 2.8.4.2[14], a plant protection product may give rise to toxic effects due to the action of toxins or co-formulants. For the assessment of such effects the following information shall be taken into consideration:

(a) studies on toxicity to arthropods;

(b) information on fate and behaviour in the various parts of the environment;

(c) available data from biological primary screening.

If mortality or signs of intoxication are observed in the tests the evaluation must include a calculation of toxicity/exposure ratios based on the quotient of the ER 50 value (effective rate) and the estimated exposure.

Also in the Commission Regulation (EU) No 546/2001, Part II, Uniform principles for evaluation and authorisation of plant protection products containing micro-organisms[14], Part C, article 2.8.4., where there is a possibility of arthropods other than bees being exposed, no authorisation shall be granted if:

(a) the micro-organism is pathogenic to arthropods other than bees,

(b) in case of toxic effects due to components in the plant protection product such as relevant metabolites/toxins, unless it is clearly established through an appropriate risk assessment that under field conditions there is no unacceptable impact on those organisms after use of the plant protection product in accordance with the proposed conditions of use. Any claims for selectivity and proposals for use in integrated pest management systems shall be substantiated by appropriate data.

The tested concentration in the effect studies is below the accumulated application rate used as worst case exposure scenario. However, it has to be kept in mind that no adverse effects were observed in the studies and therefore, the obtained margins of safety likely overestimate a possible risk for non-target arthropods by far.

[15] Commission Regulation (EU) No 546/2011: Uniform Principles for Evaluation and Authorisation of Plant Protection Products, as provided for in Article 29(6) of Regulation (EC) No 1107/2009

**Effects of Btk on Lepidoptera species in off-crop habitats**

No study on the toxicity of Btk on non-target Lepidopteran species is available. Instead data from two published reports (Broderik et al., 2006 and 2009) are summarised to support the risk assessment.

Broderick et al. (2006) investigated the impact of midgut bacteria on Btk insecticidal activity against Lepidopteran larvae. Therefore, larvae of *Lymantria dispar* were reared on diet containing a mixture of different antibiotics to destroy the natural midgut flora. Larvae reared on medium, without antibiotics, served as a control. Both groups were then administered Btk via artificial diet applied in a volume of 1 µL at standard diet disks. Larval mortality was high in the control group with 50% of the larvae found dead after Btk administration at a dose of 10 IU/µL. In the group treated with antibiotics, insecticidal activity of Btk was abolished suggesting that the larval midgut flora strongly contributes to Btk-induced mortality.

In a later study, Broderick et al. (2009) set up very similar experiments with other Lepidopteran species (*Vanessa cardui*, *Manduca sexta*, *Pieris rapae* and *Heliothis virescencs*). Insecticidal activity of Btk was determined via administration of the Btk-containing product DiPel at a rate of 25 IU/µL (first 3 species) or 100 IU/µL (*Heliothis virescencs*). Assessed was the time after which 25% or 50% of the larvae died. The time point, at which 50% mortality occurred, ranged between 1.9 and 3.04 days for the treatment with DiPel alone. As the time range for 50% mortality was in the normal range for insecticidal activity of Btk (up to 3 days) the administered dosages of 25 and 100 IU/µL can be considered as $LR_{50}$ values for the tested Lepidopteran species.

From these two studies, testing mortality of different Lepidopteran species due to Btk exposure, it can be concluded that the $LD_{50}$ of non-target Lepidopteran species is in the range of 10 to 100 IU/µL.

**Risk assessment**

To assess the risk for Lepidopteran species in off-crop habitats following the use of CoStar WG, the end-points derived from the literature are compared to off-field exposure for non-target Lepidoptera following to GAP directed use of CoStar WG using the maximum concentration in the spaying solution. As the intended maximum application rate is 2 kg/ha for use in ornamentals suspended in 500 L water, the maximum value would be $3.6 \times 10^8$ IU/L corresponding to 360 IU/µL.

To calculate the exposure in off-crop habitats, a drift of 10% is assumed according to Van de Zande et al. (2007)[16]. Off-field exposure upon a single application of CoStar WG in ornamentals would thus correspond to 36.0 IU/µL. The accumulated application rate of 12 kg/ha would correspond to 216 IU/µL in off-crop habitats.

The risk of *Bacillus thuringiensis* subsp. *kurstaki* SA-12 to non-target arthropods other than bees was assessed from margin of safety (MOS; corresponding to TER) values according to the following equation:

$$\mathrm{MOS} = \frac{\text{endpoint } [\text{IU}/\mu\text{L}]}{\text{application rate } [\text{IU}/\mu\text{L}]}$$

The resulting values for the single application rates and for the maximum application rate are presented in **Table 9.4.2-4.**

**Table 9.4.2-4 Exposure risk for non-target Lepidopteran species in off-crop habitats following GAP directed use**

| Endpoint from literature | Off-field exposure [a] | | MOS (single application / accumulated application rate) |
|---|---|---|---|
| | Single application | Accumulated application rate | |
| 10 IU/µL | 36.0 IU/µL | 216 IU/µL | 0.278 / 0.0463 |
| 25 IU/µL | 36.0 IU/µL | 216 IU/µL | 0.694 / 0.116 |
| 100 IU/µL | 36.0 IU/µL | 216 IU/µL | 2.78 / 0.463 |

[a] Assuming a drift of 10% according to Van de Zande et al. (2007)

[16] Van de Zande, J.C., J.M.G.P. Michielsen & H. Stallinga., Spray drift and off-field evaluation of agrochemicals in the Netherlands, Report 149, July 2007

The calculated Margins of Safety (MOS) vary between 0.0463 and 0.463 when the accumulated application rate is used and between 0.278 and 2.78 for a single treatment. As it is very unlikely that the same population of non-target lepidopterans will be exposed throughout all treatments the assessment for the single application can be considered more realistic. In addition, the concentration in the spraying solutions represents the worst case field and off-field exposure. It can be therefore concluded that the risk for lepidopteran species in off-crop habitats following use of CoStar WG is acceptable.

| | |
|---|---|
| RMS evaluation | In summary, the toxicity of the formulation to non-target arthropods has been investigated by carrying out Tier I tests on *Aphidius rhopalosiphi* and *Typhlodromus pyri*. Both studies confirmed the absence of toxicity of the test item to non-target arthropods. Scientific publications, show absence of adverse effects of Btk on non-target arthropods.<br><br>The beneficial organisms exposed to Btk strain SA-12 and other approved Btk strains in the referred studies are naturally occurring in the field, but they may be more important in greenhouses where they are applied for bio-control. However, they are still appropriate representatives of non-target arthropods in general and thus relevant for a risk assessment. Please refer to Vol. 3 MA, Section B.9, Point B.9.4.<br><br>To include a parameter which is relevant for the risk assessment for arthropods other than bees of a microorganism, International Units are used. International Units as a parameter for the biopotency directly reflect the insecticidal activity of a Bt strain or product and are as such considered most relevant for the risk assessment for non-target arthropods. For formal reasons the assessment based on CFU should usually be presented. However, no CFU counts have been done in available studies on NTA. Therefore, the endpoint has to be calculated based on the minimum CFU content in the product ($8.5 \times 10^{12}$ CFU/kg) whereas the exposure has to be calculated based on the maximum CFU content in the product ($5.7 \times 10^{13}$ CFU/kg). Using these values an unrealistic exposure scenario is created which overestimates the risk by far. Therefore, the RMS finds IU are more relevant for the assessment of Btk SA-12 on non-target arthropods.<br><br>For the assessment of the multiple applications the concentration of IU/µL was derived from the accumulated application rate and considering the same water volumes as for single application. This represents a complete unrealistic exposure scenario as the concentration in the tank mix will be the same at each application. It can be therefore concluded that the assessment for a single application represents the realistic worst case.<br><br>It is noted that the endpoints from the literature vary by a factor of 10 and might even vary stronger for natural populations of non-target Lepidoptera and appear to species specific. It is noted also, that insecticidal activity is only exhibited in the larvae and not in eggs, Chrysalis (pupae) or adults. So, even if the life span of a butterfly is 42 days, only part of it represents the susceptible larvae stage. Comparing the endpoints from the literature ranging between 10 and 100 IU/µL with the worst case concentration in off-crop habitats 36 IU/µL one can conclude that the risk appears to be acceptable as dilution, restricted persistence of residual insecticidal activity even on treated crops are not even considered. It can also be concluded that it is unlikely that entire natural populations will be diminished due to possible exposure to CoStar WG.<br><br>In conclusion the proposed use of CoStar WG does not pose an unacceptable risk to non-target arthropods. |

## B.9.5 Effects on earthworms

### B.9.5.1 Toxicity, infectiveness and pathogenicity in earthworms

As CoStar WG was not the representative formulation for original approval of Btk SA-12 no data on the formulation have been submitted before

**New data 2016**

It is referred to the information submitted for the MA *Bacillus thuringiensis* subsp. *kurstaki* SA-12 in Vol. 3 MA, Section B.9, Point B.9.5. The ingredients of the preparation CoStar WG, formulated as water dispersible granule, are inert and no hazards to the environment are expected (please refer to Volume 4). Therefore, studies and information on the microbial pest control agent, *Bacillus thuringiensis* subsp. *kurstaki* SA-12, are considered applicable and relevant with regard to the evaluation of effects on earthworms of the formulated products.

### B.9.5.2 Risk assessment for earthworms

**Effects on earthworms and other soil organisms**

No new studies are submitted assessing the effect of the product CoStar WG on soil organisms. Data that have already been evaluated as part of the EU review for the inclusion of this strain into Annex I, now list of approved active substances according to (EC) No 1107/2009. Please refer to the DAR 2008.

The acute toxicity of Btk SA-11 against *Eisenia fetida* has been investigated in a 14 days laboratory study. The $LC_{50}$ was determined to be above 1000 mg Delfin/kg soil dry weight. No signs of clinical toxicity or abnormal behaviour were observed. Delfin WG contains Btk SA-11 which is closely related and very similar to Btk SA-12. In addition, Delfin WG and CoStar WG are identical with regard to the product composition. The study on Delfin WG is therefore considered fully applicable to assess possible effects of CoStar WG on earthworms.

Long-term exposure of earthworms and long-term risks with respect to e.g. reproduction are considered unlikely.

**Table 9.5.2-1 Summary of the studies on effects to earthworms**

| Test substance | Test species | Endpoint | Reference |
|---|---|---|---|
| Delfin WG[1)] | *Eisenia fetida* | 1000 mg product/kg soil (dw)* | EFSA Journal 2012;10(2):2540[17] |

[1)] Delfin WG contains Btk SA-11, which is closely related and very similar to Btk SA-12
* No signs of infectivity or pathogenicity to earthworms have been observed

**Risk Assessment**

Based on the predicted environmental density in soil ($PED_{soil}$), calculated as $9.12 \times 10^8$ CFU/kg soil (dw) (corresponding to 16.0 mg product/kg dry soil; please refer to **Table 9.2.1-1**), for multiple application in ornamentals, assuming a worst case scenario that no interception and no degradation occurs between applications, the margin of safety (MOS, corresponding to TER) for earthworms (**Table 9.5.2-1**) is derived from the $LC_{50}$ value according the following formula:

$$MOS = \frac{LC_{50}[\text{mg product/kg soil dw}]}{PED_{soil}[\text{mg product/kg soil dw}]}$$

[17] 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

**Table 9.5.2-2 Exposure assessment for earthworms exposed to Btk SA-12**

| Use pattern | Test organism | $LC_{50}$ [mg product/kg soil (dw)] | $PED_{soil}$ [mg product/kg soil (dw)] | MOS |
|---|---|---|---|---|
| 6 × 2 kg product/ha in ornamentals | *Eisenia fetida* | 1000 | 16 | 62.5 |

a) Toxicity-to-exposure ratio (Trigger)

The calculated MOS value is high, indicating an acceptable acute risk to earthworms after application of CoStar WG at the maximum recommended use rate.

| | |
|---|---|
| RMS evaluation | No reports exist on any adverse effects of *Bacillus thuringiensis* to terrestrial invertebrates, and the acute toxicity study with Delfin WG (containing Btk SA-11 which is closely related and very similar to Btk SA-12) on earthworms, confirmed the absence of any adverse effects of the bacterium on terrestrial invertebrates.<br><br>In conclusion Btk SA-12 does not pose any unacceptable risk to terrestrial invertebrates including earthworms upon field application of CoStar WG. |

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

### B.9.6.1 Impact on non-target soil micro-organisms

As CoStar WG was not the representative formulation for original approval of Btk SA-12 no data on the formulation have been submitted before.

**New data 2016**

It is referred to the information submitted for the MA *Bacillus thuringiensis* subsp. *kurstaki* SA-12 in Vol. 3 MA, Section B.9, Point B.9.6. The ingredients of the preparation CoStar WG, formulated as water dispersible granule, are inert and no hazards to the environment are expected (please refer volume 4). Therefore, studies and information on the microbial pest control agent, *Bacillus thuringiensis* subsp. *kurstaki* SA-12, are considered applicable and relevant with regard to the evaluation of effects on soil micro-organisms of the formulated products.

### B.9.6.2 Risk assessment for non-target soil micro-organisms

*Bacillus thuringiensis* subsp. *kurstaki* is a native component of the soil. The bacterium has poor colonization ability and is not a good competitor in the soil. Its survival is dependent on the presence and activity of other soil microorganisms and protection from degradation effects of sunlight. Applied as a spray on above ground leaves and fruits, endospores are rapidly inactivated and δ-endotoxins are rapidly degraded when exposed to UV-radiation (EFSA Journal 2012;10(2):2540[19]).

The toxicity of Delfin WG against soil micro-organisms has been investigated in a laboratory study. 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 (please refer to the OECD Dossier, Doc IIIM, Section 6, Point 10.6). Delfin WG contains Btk SA-11 which is closely related and very similar to Btk SA-12. In addition, Delfin WG and CoStar WG are identical with regard to the product composition. The study on Delfin WG is therefore considered fully applicable to assess possible effects of CoStar WG on soil microorganisms.

**Table 9.6.2-1 Summary of the studies on effects to soil micro-organisms**

| Test substance | Test design | Endpoint | Reference |
|---|---|---|---|
| Delfin WG | C | **20 mg Delfin WG/kg soil (dw)** (corresponding to an application rate of 15 kg Delfin WG /ha) | Vol. 3 MP, Section B.9, Point B.9.5. for Btk SA-11 |
| | N | | |
| MPCP | Not stated | 0.226 µL MPCP/10 g soils, only stated as µg/10 g soil | EFSA Journal 2012;10(2):2540[18] |

C: carbon transformation, N: nitrogen turnover

**Risk assessment**

A worst-case exposure scenario was chosen that assumes complete accumulation following 6 applications at 2 kg product/ha in ornamentals. The resulting $PED_{soil}$ value was calculated to be 16 mg product/kg soil (dw) (see **Table 9.6.2-1**).

**Table 9.6.2-2 Exposure assessment for soil micro-organisms exposed to Btk SA-12**

| Use pattern | Test organism | $PED_{soil}$ [mg product/kg soil (dw)] | Endpoint [mg product/kg soil (dw)] |
|---|---|---|---|
| 6 × 2 kg product/ha in ornamentals | Soil microorganism | 16 | 20 |

*Bacillus thuringiensis* subsp. *kurstaki* had no significant effect on soil functional parameters nitrogen conversion and carbon transformation at 20 mg Delfin WG/kg soil (dw), corresponding to 15 kg product/ha. The tested concentration is higher than the maximum accumulated application rate intended for CoStar WG (12 kg/ha in ornamentals). Due to the absence of adverse effects observed in the laboratory study with Delfin WG, it can be assumed that GAP directed use of CoStar WG poses no risk for the soil microflora responsible for nitrogen conversion and carbon transformation.

| | |
|---|---|
| RMS evaluation | *B. thuringiensis* subsp. *kurstaki* is a native component of the soil. Information from public literature indicates that neither the toxins of Btk nor Btk spores and vegetative cells will act to adversely affect the activities of other native soil micro-organisms. Furthermore, it can be concluded from several studies that following application to the soil, other native soil micro-organisms utilize Btk bacteria as a source of nutrition thus, precluding growth of Btk and reducing population levels. And even when conditions are favourable for Btk to either sporulate or even initially grow, Btk populations reach a steady state as nutrients are used up and the bacteria becomes unable to compete with native soil micro-organisms, indicating that native soil micro-organisms are not adversely affected. The actual active δ-endotoxin components of Btk degrades far more rapidly in soil than Bt spores, and suggests that the spore stage is the only state in which Bt bacteria persist in natural soils.<br><br>Furthermore, the pro-toxins and toxins produced by Bt species readily and rapidly adsorb and bind to clay minerals, notably montmorillonite and kaolinite as well as to humic acids from different soils and on clay humic acid complexes. In such cases, this reduces any possible long-term interaction between the Btk toxins and other native soil micro biota, whereas free, unbound toxins are utilized as sources of nutrition.<br><br>In conclusion Btk SA-12 does not pose any unacceptable risk to soil micro-organisms upon field application of CoStar WG. |

[18] 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

## B.9.7 Additional studies

No additional studies are provided.

**Impact on Sewage systems**

According to the EFSA peer review of the risk assessment of the five Btk strains[1] a low risk is expected to methods for sewage treatment plants.

Btk does not have any bactericidal activity and was shown to have no effects on soil microbial communities. It needs to be considered that even if Btk SA-12 would reach the sewage originating either from the glasshouse or from field applications the amount of spores or cells would be very low. First of all, the product is applied as foliar spray, means that only a small fraction of the spores will reach the soil or plant growth medium environment. Mobility in soil environments is strongly restricted and leakage only takes place in the presence of a strong water flow (refer to Point 7.1.1 in the dossier). The latter, however, results in a strong dilution of the spore content eventually reaching a sewage treatment plant.

For a simple assessment the following assumptions can be made:

Worst case exposure ($PED_{Soil}$, complete leakage, no dilution, direct conversion from kg dw soil to L sewage): $1.02 \times 10^9$ CFU/L

Exposure via spray drift is covered by the soil exposure scenario (worst case $PED_{SW}$ of $1.18 \times 10^7$ CFU/L)

In comparison:

- Total cell counts in sewage (95% bacteria): > $10^{11}$ – 5 x $10^{12}$ cells/L (Franklin and Mills, 2006; McLellan et al. 2010). Blaszyk and Krzyśko-Łupicka (2013) presented an even larger diversity of microbes in sewage sludge, with municipal sludge containing bacteria at greater than $10^{16}$ CFU/g dry matter, and in sludge from the food industry the counted CFU was greater than $10^{18}$ /g d.m.

Taken into account the above mentioned numbers of bacteria in activated sludge, and assuming unrealistic worst-case conditions Btk SA-12 would always count for much less than 1% of the microorganisms already present in the activated sludge. It is unlikely that this might have any effect on the highly abundant, highly active and well adapted microbial communities in activated sludge or on the performance of the plant.

It can be therefore concluded that there are no effects on sewage treatment expected following GAP directed use of Btk SA-12.

| $PED_{Soil}$ (complete leakage, no dilution, direct conversion of kg d.w. to L sewage) | Bacterial load in activated sludge | Exposure |
|---|---|---|
| $1.02 \times 10^9$ CFU/kg d.w. or L | $10^{11}$ - $10^{12}$ cells/L, up to $10^{21}$ CFU/kg d.m. | $10^{-10}$ - 1% |

**Overall conclusion**

The above presented risk assessment proves that *Bacillus thuringiensis* subsp. *kurstaki* SA-12 and the formulated product CoStar WG are not toxic to the tested aquatic and terrestrial species, and considering the expected environmental concentration will not be hazardous to natural populations upon applications of CoStar WG following Good Agricultural Practice.

## B.9.8 References relied on

Please refer to point with References relied on in chapter B.9, in Volume 3 MA with regard to the evaluation of the literature search.

| Data point | Author(s) | Year | Title<br>Owner Report No.<br>Source (where different from owner)<br>GLP or GEP status<br>Published or not | Vertebrate study Y/N | Data protection claimed Y/N | Justification if data protection is claimed | Owner | Previous evaluation |
|---|---|---|---|---|---|---|---|---|
| KMP 9.2.1/01 | [redacted] | 2010 | COSTAR - ACUTE TOXICITY TESTING IN RAINBOW TROUT (ONCORHYNCHUS MYKISS) (TELEOSTEI, SALMONIDAE)<br>Certis USA LLC<br>[redacted]<br>Report-no.: S10-02547<br>GLP/GEP: yes<br>Published: no | yes | yes | Protected | Certis USA | New data for existing formulation, submitted for zonal authorisation in 2012 |
| KMP 9.2.2/01 | Dengler, D. | 2010 | ASSESSMENT OF TOXIC EFFECTS OF COSTAR ON DAPHNIA MAGNA USING THE 48 H ACUTE IMMOBILISATION TEST<br>Certis USA LLC<br>Eurofins Agroscience Services GmbH<br>Report-no.; S10-02549<br>GLP/GEP: yes<br>Published: no | no | yes | Protected | Certis USA | New data for existing formulation, submitted for zonal authorisation in 2012 |
| KMP 9.2.3/01 | Weber, K. | 2011 | TESTING OF EFFECTS OF COSTAR TO THE SINGLE CELL ALGA DESMODESMUS SUBSPICATUS<br>Certis USA LLC<br>Eurofins Agroscience Services GmbH<br>Report-no.: S10-02550<br>GLP/GEP: yes<br>Published: no | no | yes | Protected | Certis USA | New data for existing formulation, submitted for zonal authorisation in 2012 |
| KMP 9.3.1/01 | Vergé, E. | 2016 | DELFIN WG - ACUTE ORAL AND CONTACT TOXICITY TO THE HONEY BEE, APIS MELLIFERA L. UNDER LABORATORY CONDITIONS<br>Certis USA LLC<br>Eurofins Agroscience Services EcoChem GmbH<br>Report-no.: S15-05620<br>GLP/GEP: yes<br>Published: no | no | yes | Protected | Certis USA | New data for existing formulation, submitted for authorisation in 2016 to several MS |

| Data point | Author(s) | Year | Title<br>Owner Report No.<br>Source (where different from owner)<br>GLP or GEP status<br>Published or not | Vertebrate study Y/N | Data protection claimed Y/N | Justification if data protection is claimed | Owner | Previous evaluation |
|---|---|---|---|---|---|---|---|---|
| KMP 9.4.1/01 | Walter, C. | 2014 | COSTAR: TOXICITY TO THE PREDATORY MITE, TYPHLODROMUS PYRI SCHEUTEN (ACARI, PHYTOSEIIDAE) UNDER LABORATORY CONDITIONS<br>Certis USA LLC, S13-04875<br>Eurofins Agroscience Services EcoChem GmbH<br>GLP: yes<br>Published: no | no | yes | protected | Certis USA | New data for existing formulation, submitted for zonal authorisation in 2015 MS |
| KMP 9.4.1/02 | Walter, C. | 2016 | COSTAR WG: TOXICITY TO THE APHID PARASITOID APHIDIUS RHOPALOSIPHI DE STEFANI PEREZ (HYMENOPTERA, BRACONIDAE) UNDER LABORATORY CONDITIONS<br>Certis USA LLC, S15-01102<br>Eurofins Agroscience Services EcoChem GmbH<br>GLP: yes<br>Published: no | no | yes | protected | Certis USA | New data for existing formulation, submitted for authorisation in 2016 to several MS |
| KMP 9.4.2/01 | Broderick, N.A., Raffa, K.F., Handelsman, J. | 2006 | MIDGUT BACTERIA REQUIRED FOR BACILLUS THURINGIENSIS INSECTICIDAL ACTIVITY<br>Proc Natl Acad Sci USA, 103(41): 15196-15199<br>Report-no.: not applicable<br>GLP/GEP: no<br>Published: yes | no | no | not protected | - | New data for existing formulation, not previously submitted nor evaluated |
| KMP 9.4.2/02 | Broderick, N.A., Robinson, C.J., McMahon, M.D., Holt, J., Handelsman, J., Raffa, K.F. | 2009 | CONTRIBUTIONS OF GUT BACTERIA TO BACILLUS THURINGIENSIS-INDUCED MORTALITY VARY ACROSS A RANGE OF LEPIDOPTERA<br>BMC Biology, 7:11, 1-9<br>Report-no.: not applicable<br>GLP/GEP: no<br>Published: yes | no | no | not protected | - | New data for existing formulation, not previously submitted nor evaluated |

| Data point | Author(s) | Year | Title<br>Owner Report No.<br>Source (where different from owner)<br>GLP or GEP status<br>Published or not | Vertebrate study Y/N | Data protection claimed Y/N | Justification if data protection is claimed | Owner | Previous evaluation |
|---|---|---|---|---|---|---|---|---|
| KMP 9.7/01 1st add sub | Franklin R.B., Mills, A.L. | 2006 | STRUCTURAL AND FUNCTIONAL RESPONSES OF A SEWAGE MICROBIAL COMMUNITY TO DILUTION-INDUCED REDUCTIONS IN DIVERSITY.<br>Microbial Ecology 52(2):280-288<br>Report-no.: not applicable<br>GLP/GEP: no<br>Published: yes | no | no | not protected | - | New data for existing formulation, not previously submitted nor evaluated |
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