Appendix to:

EFSA (European Food Safety Authority), 2020. Conclusion on the peer review of the pesticide risk assessment of the active substance *Bacillus thuringiensis* subsp. *kurstaki* strain SA-11. EFSA Journal 2020;18(10):6261, 16 pp. doi:10.2903/j.efsa.2020.6261
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**Appendix A – List of end points for the active substance and the representative formulation**

| FORMAT FOR THE LISTING OF END POINTS FOR A MICROBIAL OR VIRAL PEST CONTROL AGENT (MPCA) USED IN PLANT PROTECTION |

**Identity, Biological properties, Details of uses, Further information, and Proposed Classification and Labelling**

| Active microorganism: | *Bacillus thuringiensis* subsp. *kurstaki* strain SA-11 |
|----------------------|---------------------------------------------------|
| Function (*e.g. control of fungi*): | Biological insecticide |
| Rapporteur Member State: | Denmark |
| Co-rapporteur Member State: | The Netherlands |

**Identity of the Microbial or Viral Agent used in plant protection / Active Substance** (Regulation (EU) N° 283/2013, Annex Part B, point 1)

| Name of the organism: | *Bacillus thuringiensis* subsp. *kurstaki* SA-11 |
|----------------------|---------------------------------------------------|
| Taxonomy: | 
| Domain: | Bacteria |
| Phylum: | Firmicutes |
| Class: | Bacilli |
| Order: | Bacilliales |
| Family: | Bacillaceae |
| Genus: | *Bacillus* |
| Species, subspecies, strain: | 
| Species: | *Bacillus thuringiensis* |
| Subspecies: | *kurstaki* |
| Strain: | SA-11 |

**Identification / detection:**

*Btk* SA-11 are characterized by morphological and biochemical characterization, serotyping, plasmid profiling, activity spectrum, fatty acid analysis, DNA fingerprinting AFLP and cry toxin analysis. For unequivocal identification of strain SA-11 a chromosomal primer in combination with restriction by BveI provided a specific marker.

**Culture collection:**

ARS Culture Collection (Northern Regional Research Laboratory (NRRL), at the Microbial Properties Research Unit, National Centre for Agricultural Utilization Research, Agricultural Research Service, U.S. Department of Agriculture Peoria, Illinois 61604 USA. Reference Number: NRRL B-30790.
| Minimum and maximum concentration of the MPCA used for manufacturing of the formulated product (cfu; g/kg): | Min: $8.5 \times 10^{12}$ CFU/kg MPCP (Delfin WG)  
Max: $6.4 \times 10^{13}$ CFU/kg MPCP (Delfin WG)  
Bio-potency of 32000 IU/mg (Delfin WG) |
|---|---|
| Identity and content of relevant impurities, additives, contaminating organisms in the technical grade of MPCA: | No additives, no impurities expected  
Microbial contaminant screening:  
Coliforms:  $< 10$ CFU/g  
*E. coli*: Absence in 10 g  
*Listeria*: Absence in 25 g  
*Salmonella*: Absence in 10 g  
*Shigella*: Absence in 25 g  
*Staphylococcus aureus*: Absence in 10 g  
*Vibrio cholera*: Absence in 10 g  
*Yeast and Mold*:  $< 1000$ CFU/g |
| Is the MPCA genetically modified; if so provide type of modification | Btk SA-11 is not a genetically modified strain. |
### Biological properties of the microorganism (Regulation (EU) N° 283/2013, Annex Part B, point 2)

| Origin and natural occurrence, Background level: | Btk as a species occurs naturally in a range of environmental compartments such as soils, plant surfaces and infected insects. Strain SA-11 was isolated from an infested insect. Background populations of Btk in the environment were found in the range from 10^4 to 10^6 CFU/g in soil and 0 – 10^4 CFU/g on plants in areas not previously treated with Bt. |
| --- | --- |
| Target organism(s): | Lepidopteran pests (GAP: Cydia pomonella, Spodoptera littoralis) |
| Mode of action: | The crystal proteins of *B. thuringiensis* must be ingested to be effective against the target insect. Upon ingestion of *B. thuringiensis* by the larvae, the crystalline inclusions dissolve in the larval midgut, releasing insecticidal crystal proteins. The activated Cry toxins interact with the midgut epithelium cells of susceptible insects. After binding to the midgut receptors, they insert into the apical membrane to create ion channels, or pores, disturbing the osmotic balance and permeability. This can result in colloid-osmotic lysis of the cells. Spore germination and proliferation of vegetative cells into the haemocoel may result in septicaemia, contributing to mortality of the insect larvae. |
| Host specificity: | It is generally agreed that Btk acts highly specific against members of the insect family of Lepidoptera. Some are also active against Diptera or Coleoptera. The activity spectrum of a certain strain is defined by the production of cry toxins. Btk SA-11 was shown to be active against lepidopteran species only. |
| Life cycle: | *Bacillus thuringiensis* is a ubiquitous micro-organism that colonizes a range of habitats and environments and can be found in two different stages. Under favourable conditions regarding moisture, temperature and nutrients, the basic metabolizing cell type is the vegetative cell that is actively growing and dividing. When a population of vegetative cells passes out of the exponential phase of growth, usually as a result of nutrient depletion, the differentiation of endospores begins. Endospores are formed intracellularly and are liberated after lysis of the parent cells. The transformation of dormant spores into vegetative cells can be described in three stages: (i) Activation: a reversible process that prepares the spore for germination and usually results from treatments like heating or exposure to certain chemical stimuli; (ii) Germination: the breaking of the spore stage involves the swelling, rupture of the spore coat, loss of resistance to deleterious environmental factors and increase of metabolic activity; (iii) Outgrowth: development into a vegetative cell by remerging new components from the spore coat. |
| Infectivity, dispersal and colonisation ability: | Spores are the form of Bt that assures survival. They can survive in soil for months and it was showed that cells and spores of Bt can also survive for 10 days in water, without altering their number. Applied as a spray on above ground leaves and fruits, endospores are rapidly inactivated and δ-endotoxins are rapidly degradable when exposed to UV-radiation. Neither cells nor spores of Bt are mobile, so their dispersal is limited. It is generally agreed that Bt is a poor competitor and does not germinate and grow extensively in... |
the environment. Except for target insects, Btk SA-11 is not expected to colonize any non-target organism and is not infective in humans.

| Relationships to known plant, animal or human pathogens: | As a member of the *B. cereus*-group, Btk is closely related to *B. anthracis* and *B. cereus*. Btk strains are however distinguishable from *B. cereus* and *B. anthracis*. |
|----------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Genetic stability:                                       | Culture maintenance programs ensure that only genetically unchanged and pure cultures of Btk SA-11 are used for manufacturing of the strain and the end-use product. After field or greenhouse application genetic exchange is unlikely to occur and will not lead to any adverse effects. From the literature search for Btk SA-11 it can be concluded, that transfer of genetic material cannot be completely ruled out upon use of the strain as pest control agent in agricultural settings but the likelihood is rather low because the event requires germination and growth of the applied SA-11 spores at a high level and the presence of competent recipient vegetative cells at a high level. Even under these conditions, rates of genetic exchange were shown to be extremely low. In addition, Btk SA-11 is a wild type strain and does not have the capacity to produce any other compounds than indigenous Btks already present in the environment and it is not multi-resistant. Hence, in the unlikely case that genetic material would be transferred from SA-11 to indigenous bacteria, there is no risk that any unwanted properties are spread in the environment. |
| Information on the production of relevant metabolites (especially toxins): | Btk SA-11 produces Cry1A and Cry2A insecticidal proteins and two Cry-like proteins. Apart from the Cry proteins several other insecticidal proteins are produced by Bt (vegetative insecticidal proteins VIP, cytolytic proteins Cyt etc.). Absence of toxicity to humans and mammals from all metabolites involved in the mode of action was confirmed by a literature search. Beta-exotoxins, are considered to have toxic properties but were shown not to be produced by commercial Btk strains. Btk SA-11 has the potential to form a non-haemolytic (Nhe) and haemolytic (Hbl) enterotoxin complex. The ability to produce *B. cereus*-enterotoxins and possible consequences for consumers is discussed since first evaluation of the strain. However, based on available knowledge on Btk including Btk SA-11, there is no hint that the strain has the ability to cause foodborne disease as it will not fulfil all prerequisites required for pathogenic action in humans. |
| Resistance/sensitivity to antibiotics/anti-microbial agents used in human or veterinary medicine: | Btk SA-11 has been shown to be sensitive to a broad range of antibiotics commonly used in human and veterinary medicine. The strain is not multi-resistant |
### Summary of uses supported by available data (Regulation (EU) N° 283/2013, Annex Part B, point 3)

| Crop and/or situation | Member state or Country | Product name | Product F or G or | Pest or Group of pests controlled | Preparation | Application | Application rate per treatment | PHI (days) | Remarks |
|-----------------------|-------------------------|--------------|------------------|-----------------------------------|-------------|------------|-----------------------------|------------|---------|
|                       |                         | Delfin WG    | F                | Cydia pomonella                   | Foliar spray | BBCH 67-89 | 0.0425 - 0.085               | 1000/1500  |         |
|                       |                         | Delfin WG    | G                | Spodoptera spp.                   | Foliar spray | BBCH 12-89 | 0.0425 - 0.34                | 200-1000   |         |
|                       |                         | Delfin WG    | F                | Spodoptera littoralis             | Foliar spray | BBCH 12-89 | 0.085 - 0.34                 | 500 - 1000 |         |

(a) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (e.g. fumigation of a structure) (b) Outdoor or field use (F), greenhouse use including walk-in tunnel (G) or indoor application (I) (c) e.g. biting and sucking insects, soil born insects, foliar fungi, weeds (d) e.g. wettable powder (WP), emulsifiable concentrate (EC), granule (GR) (e) CropLife International Technical Monograph no 2, 6th Edition. Revised May 2008. Catalog of pesticide. (f) All abbreviations used must be explained (g) Method, e.g. high volume spraying, low volume spraying, spreading, dusting. (h) Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plant - type of equipment used must be indicated (i) cfu = colony forming units and g/kg or g/L (j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application (k) Indicate the minimum and maximum number of applications possible under practical conditions of use (l) PHI - minimum pre-harvest interval (m) Remarks may include: Extent of use/economic importance/restrictions * based on minimum and maximum CFU content in Delfin WG # 850 g/kg or 32,000 IU/mg, (min. $8.5 \times 10^{12}$ CFU/kg, max. $6.4 \times 10^{13}$ CFU/kg)
Further information, Efficacy

Effectiveness (Regulation (EU) N° 284/2013, Annex Part A, point 6.2)

According to the latest guidance on the preparation of dossiers for the renewal of active substances, information on efficacy is not required (SANCO/10181/2013 – rev. 2.1, 13 May 2013). The representative products have all been authorised at Member State level for > 10 years and have therefore been assessed in line with Uniform Principles. The GAP for the representative uses is realistic.

Adverse effects on field crops (Regulation (EU) N° 284/2013, Annex Part A, point 6.4)

The representative products have all been authorised at Member State level for > 10 years and have therefore been assessed in line with Uniform Principles. No unacceptable adverse effects are known.

Observations on other undesirable or unintended side-effects (Regulation (EU) N° 284/2013, Annex Part A, point 6.5)

The representative products have all been authorised at Member State level for > 10 years and have therefore been assessed in line with Uniform Principles. No unacceptable side effects are known.

Classification and proposed labelling (Symbol, Indication of danger, Risk phrases, Safety phrases)

| with regard to physical/chemical data: | Not required |
|--------------------------------------|-------------|
| with regard to toxicological data:    |             |
| H319: Causes serious eye irritation  |             |
| P101: If medical advice is needed, have product container or label at hand |             |
| P102: Keep out of reach of children  |             |
| P264: Wash hands and face thoroughly after handling* |             |
| P280: Wear eye/face protection*      |             |
| P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing. |             |
| P337+P313: If eye irritation persists: Get medical advice/attention* |             |
| P501: Dispose of the container/contents in accordance with municipal rules for disposal of waste |             |
| *Based on H319, P264, P280 and P337+P313 are not highly recommended, but can be included. |
| Safety precaution phrases:           |             |
| Contains *Bacillus thuringiensis* subsp. *kurstaki* SA-11; microorganisms may have the potential to provoke sensitising reactions. |             |
| Keep away from food, drink and animal feeding stuffs. |             |
| with regard to fate and behaviour:   | Not required |
|                                       |             |
with regard to ecotoxicological data: Not required

**Methods of analysis** (Regulation (EU) N° 283/2013, Annex Part B, point 4 and Regulation (EU) N° 284/2013, Annex Part B, point 5)

**Analytical methods for the microorganism** (MA 4.1 & MP 5.1)

| Manufactured microorganism (principle of method): | Biopotency (bioassay with *T. ni*), CFU (validated method) |
|-----------------------------------------------|-------------------------------------------------------------|
| Impurities and contaminating microorganisms in manufactured material (principle of method): | Standard microbiological methods for detection of microbial contaminants |
| Microbial Pest Control Product (principle of method): | See above |

**Analytical methods for residues (viable and non-viable) in exposed compartments and organisms** (MA 4.2 & MP 5.2)

| of the active microorganism (principle of method): | Specific markers are available to monitor the strain in agricultural fields. |
|---------------------------------------------------|-------------------------------------------------------------------------|
| of relevant metabolites (principle of method):     | Cry1Ab Soil: extraction with phosphate buffered saline Tween, quantification with commercial ELISA kit. LOQ 0.25 ng/mL. Water: processing via lyophilisation and filter centrifugation, quantification with ELISA. Method detection limit 2.1 ng/L. |

**Impact on Human and Animal Health** (Regulation (EU) N° 283/2013, Annex Part B, point 5 and Regulation (EU) N° 284/2013, Annex Part B, point 7)

| Medical data: (including medical surveillance on manufacturing plant personnel) (MA 5.1.1) | There are no confirmed case reports linking agricultural use of plant protection products based on Btk strains with human disease although Btk products have been used worldwide for more than sixty years. No incidents related to adverse health effects such as toxicological effects, allergic response, or irritation, to employees, resulting from exposure to *B. thuringiensis* subsp. *kurstaki* SA-11 during development, manufacture, preparation or field application of the product have been reported |
| Sensitisation: (MA 5.2.1 & MP 7.2.3) | In a 3-year follow up study on sensitization and health effects of exposure to microbiological control agents used in Danish greenhouses including *Bacillus thuringiensis* subsp. *kurstaki*, increased IgE levels was observed in 53% of the blood samples (measurement was only qualitative – a positive IgE was defined as exceeding the detection limit of 0.025 OD units). *Bacillus thuringiensis* subsp. *kurstaki* strain SA-11 should be regarded as potential sensitizers and the following warning phrase is proposed: “*Bacillus thuringiensis* subsp. *kurstaki* strain SA-11. Micro-organisms may have the potential to provoke sensitising reactions” |
| Acute oral infectivity, toxicity and pathogenicity: (MA 5.2.2.1 & MP 7.1.1) | No signs of toxicity, pathogenicity or infectivity have been detected upon single oral exposure to Btk SA-11 (Delfin WG formulation). LD$_{50}$ > 5000 mg /kg bw corresponding to 1.12 × 10$^{11}$ CFU Btk SA-11/ kg bw |
## Acute intratracheal/inhalation infectivity, toxicity and pathogenicity:

| Description | Details |
|-------------|---------|
| LC₅₀ | > 1.00 mg/L (3.4 x 10⁷ CFU Btk SA-11/L) |

## Acute intravenous/intraperitoneal infectivity:

| Description | Details |
|-------------|---------|
| No pathogenicity upon pulmonary exposure observed |

## Genotoxicity:

| Description | Details |
|-------------|---------|
| Mouse micronucleus study (intraperitoneal administration) with the spore-crystal complex containing Cry1Aa and Cry1Ac, Cry1Ab and Cry2Aa: equivocal results. Data gap: unclear whether toxins had been solubilised/activated prior to administration or not. Possible concern for non-dietary routes but not for dietary route. Data is lacking for Cry 2Aa and Cyt. |

## Cell culture study:

| Description | Details |
|-------------|---------|
| Not required. |

## Information on short-term toxicity and pathogenicity:

| Description | Details |
|-------------|---------|
| 3-week study in rats did not reveal any indications of direct toxicity, infectivity or pathogenicity NOAEL 10⁶ CFU per animal per day. No adverse effects observed. Inhalation 14-day mouse study conducted with formulated Btk (Dipel): LOAEL 4.2 x 10⁴ CFU/mouse based on patches of interstitial lung inflammation in 18% of animals at 70 days. NOAEL: not established (data gap). Full clearance observed at 70 days. |

## Dermal toxicity:

| Description | Details |
|-------------|---------|
| Delfin WG (containing Bacillus thuringiensis subsp. kurstaki strain SA-11/bag laboratory: 9.1 x 10¹⁰ CFU/g): Rabbit: LD₅₀ > 20 mg/kg bw (3.5 x 10⁹ CFU/site) |

## Specific toxicity, pathogenicity and infectivity:

| Description | Details |
|-------------|---------|
| Dermal toxicity study with 4 mL Javelin WG SAN 415 WG 354 (corresponding to 40 mg test item): negative. Eye irritation: Delfin WG/ Javelin WG revealed conjunctival irritation that was completely reversible within 14 days. The hazard statement H319: Causes serious eye irritation is required |

## Genotoxicity – in vivo studies in germ cells:

| Description | Details |
|-------------|---------|
| No studies conducted with Btk. |

## Reference values

| Description | Details |
|-------------|---------|
| AOEL: | As no exposure models exist for microbials, setting an AOEL would be of low relevance to the risk assessment. The recommended use of RPE for both operators and workers is considered to cover the potential risk after repeated exposure by inhalation. |

| Description | Details |
|-------------|---------|
| ADI: | The threshold of 10⁵ CFU/g food is applicable to cover the risk of food-borne poisonings caused by the B. cereus group of micro-organisms. |

| Description | Details |
|-------------|---------|
| ARfD: | The threshold of 10⁵ CFU/g food is applicable to cover the risk of food-borne poisonings caused by the B. cereus group of micro-organisms. |

## Exposure (operator, workers, bystander, consumer)

| Description | Operators | Workers | Bystanders |
|-------------|-----------|---------|------------|
| Operators: | No risk is anticipated. PPE (coveralls and gloves) and RPE (disposable filtering face piece respirator to at least EN149 FFP3 or equivalent) are recommended. |
| Workers: | Transplantation or handling of pots: PPE (coveralls and gloves) and RPE is recommended. |
| Bystanders: | Not applicable |
Residues (Regulation (EU) No 283/2013, Annex Part B, point 6 and Regulation (EU) No 284/2013, Annex Part B, point 8)

| Viable residues: B. thuringiensis ssp. kurstaki | B. thuringiensis ssp. kurstaki spores are not persistent on crop, half-life less than 1 day, nevertheless a storage stability of Bacillus thuringiensis subsp. kurstaki strain SA-11 in high-water commodities is still required (data gap). Based on uncertainties in the toxicology section related to potential production of enterotoxin in the human gut, a threshold level of $10^5$ CFU/g at harvest was proposed. Therefore, quantification of viable counts linked to specific PHIs is requested (data gap). |
| Non-viable residues: | Not relevant for dietary exposure (see Section 2). |

Fate and Behaviour in the Environment (Regulation (EU) No 283/2013, Annex Part B, point 7 and Regulation (EU) No 284/2013, Annex Part B, point 9)

| Persistence and multiplication (competitiveness) in soil, water and air: Btk SA-11 | Btk SA-11 Soil: Bacillus thuringiensis including Btk SA-11 occurs naturally and ubiquitously in the environment. It is a common component of the soil micro-biota and has been isolated from most terrestrial habitat. Available information indicates that Bacillus thuringiensis spores may persist from days to years in soil under natural field conditions. The low potential for spore germination, growth and re-sporulation in bulk soils minimises multiplication. Germination in the rhizosphere may occur. |
| Water: Information on B. thuringiensis Btk SA-11 was not available. Bacillus thuringiensis including Btk is an inhabitant of aquatic environments. Data gap for information on proliferation in natural surface water systems. Air: re-aerolisation of applied spores is possible but spores rapidly drop in viability following release to air. Fate and transport via air after application is unlikely to play a role in environmental exposure to B. thuringiensis subsp. kurstaki including Btk SA-11 spores and endotoxins. Endotoxins Soil: Various studies on persistence of Bt crystalline proteins are available. The agreed degradation endpoints for modelling purposes are: DT50 soil = 41.3 d |
| The following tables contain degradation data taken from the Renewal Assessment Report of Bacillus thuringiensis subsp. Aizawai strain GC-91 Volume 3 MA-B8. |

| DT50 Soil (days) | Experiment/Cry Protein |
|-----------------|------------------------|
| 0.0208          | Free protoxin/toxin    |
| 0.1667          | Free protoxin/toxin    |
| 2.7             | 14C labelled protoxins, sterilised, amended soil |
| 5.2             | 14C labelled protoxins, sterilised, amended soil |
| 3               | Natural soil           |
| 21              | Natural soil           |
| 7               | Natural soil (laboratory) |
| 15              | [14C]Cry1Ac Natural soil (laboratory) |
| 9.8             | Cry1Ab and Cry1Ac Natural soil (laboratory) |
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Water: The agreed degradation endpoints for modelling purposes are:

DT50 whole water sediment system = 28 d

The following tables contain degradation data taken from the Renewal Assessment Report of *Bacillus thuringiensis* subsp. *aizawai* strain GC-91 Volume 3 MA-B8.

| Experiment/Cry Protein                           | DT50 Whole water sediment system (days) |
|-------------------------------------------------|-----------------------------------------|
| Bti protoxin, laboratory microcosms             | 0.9                                     |
| Bti protoxin, laboratory microcosms             | 1.5                                     |
| Bti protoxin river                              | 7                                       |
| Bti protoxin river                              | 28                                      |
| Cry 1 Ac artificial ‘natural’ water             | 12.8                                    |
| Cry 1 Ac artificial ‘natural’ water, sterile hydrolysis | 130.8                     |
| Cry 1 Ac artificial ‘natural’ water, sterile hydrolysis | 93.7                              |

Mobility:

Btk SA-11

Mobility of spores of *B. thuringiensis* including Btk SA-11 can be considered limited.

Endotoxins
Cry proteins are adsorbed by soil and exhibit low mobility after their release into soil. The agreed mobility endpoints for modelling purposes are: K\text{doc} = 1000\,\text{mL/g} 
1/n = 1

The following tables are taken from the Renewal Assessment Report of Bacillus thuringiensis subsp. azawai strain GC-91 Volume 3 MA-B8.

### Maximum and minimum values (average in brackets) of some soil chemical and physical properties

| All soil samples | Clay content / g kg\(^{-1}\) | Corg content / g kg\(^{-1}\) | pH (H\(_2\)O) | CEC / cmolc kg\(^{-1}\) | Corg : clay / % |
|------------------|-------------------------------|-------------------------------|----------------|------------------------|----------------|
| (n=41) Range (average) | 16–707 (249) | 0.6–243 (38) | 4.3–8.6 (6.2) | 0–39 (11.4) | 1–70 (17) |
| Soils under cereal culture (n=16) Range (average) | 78–480 (247) | 6.9–33 (16.8) | 4.6–82 (6.5) | 2.3–31.6 (12.9) | 3–22 (8) |
| Soils from (semi-) natural systems (n=25) Range (average) | 16–707 (250) | 0.59–243 (51) | 4.3–8.6 (6.1) | 0–39 (10.5) | 1–70 (23) |
| Subset of soils studied for all proteins (n=19) Range (average) | 16–707 (295) | 0.59–243 (45) | 4.3–8 (6.3) | 0–39 (12.6) | 1–58 (17) |

### Minimum and maximum values (average in brackets) of affinity (Kd / dm\(^3\) kg\(^{-1}\)) for each of the proteins in the soil samples

| Protein | Cr1Ac | Cry2A | Cry1C |
|---------|-------|-------|-------|
| Full sample set (n=41) Range (average) | 1630–38 400 (12 100) | | |
| Soils under cereal culture (n=16) Range (average) | 1630–28 600 (10 100) | | |
| Soils under (semi-) natural land use (n=25) Range (average) | 2820–38 400 (13 200) | | |
| Soils studied for all proteins (n=19) Range (average) | 1630–24 400 (11 300) | 1560–29 300 (16 100) | 837–54 600 (18 300) |
| Soils under cereal culture (n=7) Range (average) | - | 1550–26 700 (4700) | 5000–54 600 (19 150) |
| Soils under (semi-) natural land use (n=12) Range (average) | - | 1560–29 300 (13 700) | 837–42 900 (17 700) |
PECsoil (Predicted Environmental Concentration) micro-organisms

For the calculation the content of 850 g a.s./kg product has been considered. In addition, the PEC value is indicated in CFU/kg soil dry weight (dw), based on a maximal content of $6.4 \times 10^{13}$ CFU/kg.

Assumptions:
Application rate Delfin WG:
2 kg product/ha (equivalent to 1700 g a.s./ha or $1.28 \times 10^{14}$ CFU/ha based on max. content)
Accumulated application rate (up to 6 treatments): 12 kg product/ha, equivalent to 10200 g a.s./ha or $7.68 \times 10^{14}$ CFU/ha

Incorporation into the top 5 cm layer (resulting soil volume $V = 0.05 \text{ m} \times 10,000 \text{ m}^2 = 500 \text{ m}^3$)

Soil density $\rho$ of 1.5 g/cm$^3$ ($= 1.5 \times 10^3$ kg/ m$^3$)

Soil mass / ha: $V \times \rho = 750,000$ kg soil dry weight

Calculation of the predicted environmental concentration of Delfin WG and *B. thuringiensis* in soil (PECsoil) after 6 applications at 2 kg Delfin WG/ha in turf

| Accumulated application rate [kg product/ha] | Rate [mg product/m²] | Soil depth [cm] | Bulk density [g/cm³] | Initial PEC related to soil depth [mg product/kg soil (dw)] |
|---------------------------------------------|----------------------|-----------------|----------------------|------------------------------------------------------------|
| 12                                          | 1200                 | 5.00            | 1.5                  | 16.0                                                        |
| Accumulated application rate [kg a.s./ha]   | Rate [mg a.s./m²]    | Soil depth [cm] | Bulk density [g/cm³] | Initial PEC related to soil depth [mg a.s./ kg soil (dw)]  |
| 10.2                                        | 1020                 | 5.00            | 1.5                  | 13.6                                                        |
| Accumulated application rate [CFU/ha]       | Rate [CFU/m²]        | Soil depth [cm] | Bulk density [g/cm³] | Initial PEC related to soil depth [CFU/ kg soil (dw)]      |
| $7.68 \times 10^{14}$                       | $7.68 \times 10^{10}$| 5.00            | 1.5                  | $1.02 \times 10^9$                                         |

PECsoil Cry proteins

Based on the average total Cry-proteins in the technical active substance of 11.8%, and a content of technical grade active substance in the product of 85%, a PEC in soil for Cry-proteins in soil may be estimated.

| Accumulated application rate [kg product/ha] | Rate [mg product/m²] | Soil depth [cm] | Bulk density [g/cm³] | Initial PEC Cry related to soil depth [mg /kg soil (dw)] |
|---------------------------------------------|----------------------|-----------------|----------------------|----------------------------------------------------------|
| 12                                          | 1200 (120 mg Cry)    | 5.00            | 1.5                  | 16 mg/kg product (1.6 mg/kg Cry protein)                 |

PECsw micro-organisms
Calculation of the predicted environmental concentration of Delfin WG and *B. thuringiensis* in lentic water bodies (PEC<sub>sw</sub>) after 6 applications at 1 kg Delfin WG/ha in pome fruits

|                          | Application rate<sup>1)</sup> | Relevant drift rate [%]<sup>2)</sup> | Amount reaching the water | Water volume (30 cm water layer) | Initial PEC<sub>sw</sub> |
|--------------------------|------------------------------|----------------------------------|--------------------------|---------------------------------|--------------------------|
| **Delfin WG**            | 6 kg/ha                      | 9.21                             | 55.26 mg/m<sup>2</sup>   | 300 L/m<sup>2</sup>             | 184.2 µg/L               |
| **Bacillus thuringiensis**<br>subsp. kurstaki SA-11 | 5.1 kg/ha                    | 9.21                             | 46.97 mg/m<sup>2</sup>   | 300 L/m<sup>2</sup>             | 156.6 µg/L               |
|                          | 3.84 × 10<sup>14</sup> CFU/ha|                                  | 3.54 × 10<sup>9</sup> CFU/m<sup>2</sup> |                                  | 1.18 × 10<sup>7</sup> CFU/L |

<sup>1)</sup> Accumulated application rate, assuming no degradation between applications<br>
<sup>2)</sup> Drift value for 6 applications in fruit crops (late)

Calculation of the predicted environmental concentration of Delfin WG and *B. thuringiensis* in lentic water bodies (PEC<sub>sw</sub>) after 6 applications at 2 kg Delfin WG/ha in turf

|                          | Application rate<sup>1)</sup> | Relevant drift rate [%]<sup>2)</sup> | Amount reaching the water | Water volume (30 cm water layer) | Initial PEC<sub>sw</sub> |
|--------------------------|------------------------------|----------------------------------|--------------------------|---------------------------------|--------------------------|
| **Delfin WG**            | 12 kg/ha                     | 1.64                             | 19.68 mg/m<sup>2</sup>   | 300 L/m<sup>2</sup>             | 65.6 µg/L               |
| **Bacillus thuringiensis**<br>subsp. kurstaki SA-11 | 10.2 kg/ha                   | 1.64                             | 16.73 mg/m<sup>2</sup>   | 300 L/m<sup>2</sup>             | 55.76 µg/L               |
|                          | 7.68 × 10<sup>14</sup> CFU/ha|                                  | 1.26 × 10<sup>9</sup> CFU/m<sup>2</sup> |                                  | 4.2 × 10<sup>6</sup> CFU/L |

<sup>1)</sup> Accumulated application rate, assuming no degradation between applications<br>
<sup>2)</sup> Drift value for 6 applications in field crops

**PEC<sub>sw</sub> Cry-proteins**

Method of calculation

FOCUS Steps 1 and 2 calculations using “STEPS 1-2 in FOCUS” calculator, ver 3.2.
- Pome / stone fruit early applications
- Soil DT<sub>50</sub> 41.3 days
- Water system DT<sub>50</sub> 28 days
- Water DT<sub>50</sub> 11 days
- sediment DT<sub>50</sub> 96 days
- Soil adsorption K<sub>DOC</sub> 1000 mL/g

Application rate

6 x 0.1 kg Cry protein/ha (based on a crystalline protein content of 11.8% with 85% TGAI in product) with spray interval of 7 days.

**Predicted environmental concentrations of Cry-proteins in the water body (surface water and sediment) – pome fruit (worst case exposure; FOCUS surface water)**

| Scenario                          | Step 1               | Step 2               |
|-----------------------------------|----------------------|----------------------|
|                                   | PEC<sub>sw</sub> actual (µg/L) | PEC<sub>sed</sub> (µg/kg) | PEC<sub>sw</sub> actual (µg/L) | PEC<sub>sed</sub> (µg/kg) |
| Pome/stone fruit early application NE | 144.11               | 857.14               | 28.25                  | 240.70           |
| Pome/stone fruit early application SE | 144.11               | 857.14               | 35.5462               | 313.16           |
PECgw Cry-proteins

Predicted environmental concentrations of Cry-proteins in groundwater – grass (worst case exposure, FOCUSPEARL 4.4.4)

Method of calculation

Soil DT$_{50}$ 41.3 days
Soil adsorption $K_{d,soil}$ 1000 mL/g $1/n=0.9$

Application rate

6 x 0.2 kg Cry protein/ha (based on average total crystalline protein content of 11.8% with 85% TGAI in product) with spray interval of 7 days.

| Scenario          | Grass Conc ug/L |
|-------------------|-----------------|
| All scenarios     | <0.001          |

Effects on non-target organisms (Regulation (EU) N° 283/2013, Annex Part B, point 8 and Regulation (EU) N° 284/2013, Annex Part B, point 10)

Effects on birds and mammals

| Application rate (g a.s./ha) | Test substance | Crop | Category (e.g. insectivorous bird) and species | Time-scale | Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects) |
|------------------------------|----------------|------|---------------------------------|------------|----------------------------------------------------------------------------------|
| 850 - 1700                   | SAN 415 I Technical (Btk SA-11) | Pome fruits, tomato, turf, sports | Acute oral, Bobwhite quail | 5-d observation | Btk SA-11: LD$_{50}$ > 5.3 x 10$^8$ CFU/kg bw/day (22 mg/kg b.w.) |
|                              | SAN 415 WG formulation (Btk SA-11) (Javelin-R/Delfin-R) | | Acute oral, Rat | 14-d observation | Delfin: LD$_{50}$ > 5 g Delfin /kg bw (LD$_{50}$ > 1.12 x 10$^{11}$ CFU/kg bw) (4250 mg a.s./kg bw) |
|                              | Delfin WG | | Acute oral, Mouse | 14-d observation | Delfin WG: LD$_{50}$ > 5 g Delfin WG/kg bw (LD$_{50}$ > 2.4 x 10$^{11}$ CFU/ kg bw) (4250 mg a.s./kg bw) |

Effects on aquatic organisms

| Group | Test substance | Time-scale | Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects) |
|-------|----------------|------------|----------------------------------------------------------------------------------|
| Fish species (specify): Oncorhynchus mykiss | SAN 415 I (Btk SA-11) technical powder | 30-d (semi-static) | Btk SA-11: LC$_{50}$ > 41.5 mg/L$^b$ |
|       |                 |            | LC$_{50}$ > 1.0 x 10$^9$ CFU/L                                                    |
### Invertebrate species

| Invertebrate species | Test substance | Route/timescale | Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects) |
|----------------------|----------------|----------------|----------------------------------------------------------------------------------|
| *Daphnia magna*      | SAN 415 I (Btk SA-11) technical powder | 48-h (static) | Btk SA-11:  
EC$_{50}$ > 41.5 mg/L  
EC$_{50}$ > 1.0 × 10$^9$ CFU/L |

### Effects on algae:

(Species, growth, growth rate, capacity to recover)

| Species | Test substance | Route/timescale | Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects) |
|---------|----------------|----------------|----------------------------------------------------------------------------------|
| *Selenastrum capricornutum* | SAN 415 I (Btk SA-11) technical powder | 72-h (static) | Btk SA-11:  
EC$_{50}$ > 41.5 mg/L  
EC$_{50}$ > 1.0 × 10$^9$ CFU/L |

### Effects on aquatic plants

(Species, growth, growth rate, capacity to recover)

Not toxic, pathogenic or infective to aquatic plants based on available experience with Btk SA-11 products and efficacy testing.

### Effects on bees

| Species | Crop | Test Substance | Route/time-scale | Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects) |
|---------|------|----------------|-----------------|----------------------------------------------------------------------------------|
| *Apis mellifera* | Pome fruits, tomato, turf, sports | Delfin WG | Oral/15-d | LD$_{50}$ > 82 μg product/bee or > 4.2 × 10$^6$ CFU/bee |
|          |      |                | Contact/19-d    | LD$_{50}$ > 100 μg product/bee or > 5.1 × 10$^6$ CFU/bee |

**Laboratory Test Data gap** for the potential effects to honeybee larvae, infectivity and pathogenicity to honeybee Apis mellifera

| Species | Stage | Test Substance | Dose | Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects) |
|---------|-------|----------------|------|----------------------------------------------------------------------------------|
| *Aphidius rhopalosiphi* | Adult | Delfin WG | Acute laboratory (glass plate), 4.5 kg product/ha | EC$_{50}$ > 4.5 kg product/ha |
| *Typhlodromus pyri* | Protonymphs | Delfin WG | Acute laboratory (glass plate), 4.5 kg product/ha | EC$_{50}$ > 4.5 kg product/ha |
Effects on other terrestrial invertebrates

| Toxicity, infectivity and pathogenicity: | *Eisenia fetida* |
| (endpoint, value or other description of effects) | Delfin WG |
| | No signs of toxicity, infectivity or pathogenicity at 1000 mg product/kg soil (dw) |

Further information: 

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Effects on soil microorganisms

Delfin WG has no significant effects on the nitrogen turnover and short-term respiration activity of soil microflora at tested concentrations of up to 20.0 mg/kg soil (dw), equivalent to 15 kg Delfin WG/ha

Additional studies

None