Appendix to:
EFSA (European Food Safety Authority), 2020. Conclusion on the peer review of the pesticide risk assessment of the active substance *Bacillus thuringiensis* ssp. *israelensis* (serotype H-14) strain AM65-52. EFSA Journal 2020;18(12):6317, 17 pp. doi:10.2903/j.efsa.2020.6317
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**Appendix A – List of end points for the active substance and the representative formulation**

**Identity, Biological properties, Details of uses, Proposed Classification and Labelling, and Analytical methods**

| Active micro-organism | *Bacillus thuringiensis* ssp. *israelensis* (serotype H-14) strain AM65-52 |
|-----------------------|--------------------------------------------------------------------------|
| Function (e.g. fungicide) | Insecticide |
| Rapporteur Member State | Sweden |
| Co-rapporteur Member State | Spain |

**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* ssp. *israelensis* (serotype H-14) strain AM65-52 |
|----------------------|--------------------------------------------------------------------------|
| Taxonomy             | Domain: Bacteria  
                       | Phylum: Firmicutes  
                       | Class: Bacilli  
                       | Order: Bacilliales  
                       | Family: Bacillaceae  
                       | Genus: Bacillus |
| Species, subspecies, strain | Species: *Bacillus thuringiensis*  
                             | Subspecies: *israelensis*  
                             | Strain: AM65-52 |
| Identification / Detection | No strain specific method has been submitted |
| Culture collection | American Type Culture Collection no.: ATCC – 1276 |
| Minimum and maximum concentration of the MPCA used for manufacturing of the formulated product (cfu; g/kg) | Continuous manufacturing process, therefore specification set on MPCI:  
1.2 x 10^{13} CFU/L (Gnatrol SC)  
Min: 5.55 x 10^{12} CFU/L MPCP (Gnatrol SC)  
Max: 2.5 x 10^{13} CFU/L MPCP (Gnatrol SC)  
Bio-potency of 1200 ITU/mg (Gnatrol SC) |
| Identity and content of relevant impurities, additives, contaminating organisms in the technical grade of MPCA (cfu; g/kg) | Contains 1,2-benzisothiazol-3(2H)-one (BITT). |
| Is the MPCA genetically modified; if so provide type of modification | No |
### Biological properties of the microorganism

**Origin and natural occurrence,**

*Bacillus thuringiensis* subsp. *israelensis*, Bti, is a common naturally occurring soil micro-organism with worldwide distribution. Bti is also frequently isolated in freshwater and have been isolated from intertidal brackish sediments in mangroves. A sample collected from the edge of the pool in a dried river-bed in the north central Negev Desert, near Kibbutz Zeelim, containing dead and decomposing larvae, water and silty mud was taken to the laboratory and refrigerated. Bacteria were isolated from this sample and purified to a single colony designated ONR 60A from which the strain AM65-52 derives.

**Background level**

Not reported.

**Target organism(s)**

Larvae of fungus gnats (*Diptera sciaridae*).

**Mode of action**

The mode of action of Bti and its toxins in the target insects vary depending on insect species. Some insects require both spores and toxins for mortality.

*B. thuringiensis* forms parasporal crystalline inclusions which under the alkaline conditions in the gut of the target organisms are activated and can bind to midgut cell membranes of susceptible insects. This results in ion channels, or pores, disturbing the osmotic balance and permeability resulting in the cells swelling, cell lysis and eventual insect death. The active organism produces the delta-endotoxins Cry4Aa, Cry4Ba, Cry10A, Cry11A, Cyt1Aa, Cyt2Ba, and Cyt1Ca.

Spore germination and proliferation of the vegetative cell into the haemocoel may result in septicaemia, contributing to mortality of the insect larvae. Insect susceptibility to Bt toxins is also influenced by insect midgut bacteria and that their importance vary depending on insect species.

**Host specificity**

Bti affects species of the genera Diptera, Hemiptera, Homoptera, Coleoptera, Lepidoptera and Trematoda. The strain-specific host-range of the active organism is unclear.

**Life cycle**

*Bacillus thuringiensis* cultures are found in nature in one of two states, either as vegetative cells that are actively growing and dividing, or as spores. The spores are a resistant, metabolically inactive, resting form with a completely different fine structure, chemical composition and enzymatic constitution from the vegetative cells. The life-cycle of *Bacillus thuringiensis* follows the characteristic process of spore formation (sporulation) typical of *Bacillus* cultures, with the exception that insect toxin containing parasporal bodies are formed during sporulation. Spore formation normally commences when vegetative growth ceases due to a lack of nutrients or a shift in the environment to conditions less favourable for vegetative growth. No information was provided on generation time or host organism (data gap).

**Infectivity, dispersal and colonisation ability**

**Infectivity:**

Measurements of infectivity in target insects have not been provided. For infectivity in humans, see section 2 below.

**Dispersal**

*B. thuringiensis* subsp. *israelensis* spores are suggested to be rather immobile after their introduction into the environment.

**Optimum environmental conditions for growth (including temperature)**

The available information regarding environmental conditions for Bti AM65-52 is not considered sufficient (data gap).

**Colonisation ability**

Recycling of Bti in the field seems to be possible, but long-term effects of field Bti application on the abundance of Bti in the environment seem to be moderate.
### Pathogenicity:

Spore germination and proliferation of the vegetative cell into the haemocoel of the target organisms may result in septicaemia, contributing to mortality of the insect larvae.

### Relationships to known plant, animal or human pathogens

*B. thuringiensis* is a member of the *Bacillus cereus* group which comprises closely related Gram positive bacteria, including two human pathogenic species, *B. cereus* and *B. anthracis*. *B. cereus* can cause food poisoning by the production of toxins, whereas *B. anthracis* is the causative agent of anthrax.

### Genetic stability

Bt strains have the ability to act as donors as well as recipients of genetic material. This transfer has been shown to occur *in vitro* (laboratory cultures (broth), milk, rice pudding, river water) and *in vivo* (gnotobiotic rats and dipteran larvae). Plasmid transfer between two Bti strains was shown and Bt was able to have a full life cycle (germination of spores, several cycles of growth, and sporulation of vegetative cells) in the intestine of gnotobiotic rats.

Based on the information above, genetic transfer in soil could theoretically occur. However, since there is no concern regarding antibiotic resistance for the organism this was not considered as an issue of concern.

### Information on the production of relevant metabolites (especially toxins)

The active organism produces the delta-endotoxins Cry4Aa, Cry4Ba, Cry10A, Cry11A, Cyt1Aa, Cyt2Ba, and Cyt1Ca. Sufficient information has not been provided regarding which vip and sip proteins that are produced by the active organism (*data gap*). Enterotoxins and beta-exotoxins are not considered to be of concern for the representative use.

### Resistance/ sensitivity to antibiotics / anti-microbial agents used in human or veterinary medicine

Antimicrobial susceptibility of the *Bacillus thuringiensis* ssp. *israelensis* (serotype H-14) strain AM65-52 was tested against 15 antibiotics: gentamicin, kanamycin, streptomycin, neomycin, tetracycline, erythromycin, clindamycin, chloramphenicol, ampicillin, vancomycin, quinupristin and dalfopristin combination, linezolid, trimethoprim, ciprofloxacin, rifampicin. The strain was susceptible to all the antibiotics tested except trimethoprim. It should be noted however, that literature data showed resistance of *B. thuringiensis* ssp to the beta-lactams: penicillin, ampicillin and cephalothin.
Summary of uses supported by available data
(Regulation (EU) N° 283/2013, Annex Part B, point 3)

| Crop and/or situation (a) | Zone or Member State | Product name/code | F, G or I (b) | Pests or Group of pests controlled (c) | Formulation (d) (f) | conc. of as (i) | Application method kind (f-h) | growth stage & season (j) | number min-max (k) | interval between applications (min) (days) | Application rate per treatment | PHI (days) (l) | Remarks: (m) |
|---------------------------|----------------------|-------------------|--------------|---------------------------------------|---------------------|----------------|-------------------------------|---------------------------|----------------|--------------------------------------|-----------------------------|----------------|----------------|
| Ornamental plants         | EU                   | Gnatrol® SC       | G            | Fungus gnats                          | SC                  | 123 g/l (11.61% w/w) Bacillus thuringiensis subsp. israelensis (serotype H-14, strain AM 65-52) with a nominal potency of 1200 ITU/mg. | Drench via a sprayer or irrigation | All growth stages | 3 | 3-7 days | 0.246 kg a.s./hL – 0.03075 kg a.s./hL | 0.5–2 l/m² or 5 000 to 20 000 l/ha | 6.15 kg to 12.30 kg MPCA/ha | (50 to 100 l product/ha or 5 ml to 10 ml product/m²) 9.1–18.2×10¹¹ ITU/ha Approx 1×10¹⁴ CFU/ha | 0 | The applicant has confirmed that the intended use is on soil bound systems in permanent greenhouses. |

Remarks:
(a) For crops, the EU and Codex classifications (both) should be taken into account; where relevant, the use situation should be described (e.g. fumigation of a structure)
(b) Outdoor or field use (F), greenhouse application (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) GCPF Codes - GIFAP Technical Monograph No 2, 1989
(f) All abbreviations used must be explained
(g) Method, e.g. high volume spraying, low volume spraying, dusting, drench
(h) Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plant-type of equipment used must be indicated
(i) 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) The minimum and maximum number of application possible under practical conditions of use must be provided
(l) PHI – minimum pre-harvest interval
(m) Remarks may include: Extent of use/economic importance/restrictions

Microbial Pest Control Agent
Classification and proposed labelling (Symbol, Indication of danger, Risk phrases, Safety phrases)

| Category                                      | Details                                                                 |
|-----------------------------------------------|------------------------------------------------------------------------|
| with regard to physical/chemical data         | Not applicable                                                         |
| with regard to toxicological data             | Contains *Bacillus thuringiensis* ssp. *israelensis*. Microorganisms may have the potential to provoke sensitising reactions. EUH208 - Contains 1,2-benzisothiazol-3(2H)-one (BIT). May produce an allergic reaction. |
| with regard to fate and behaviour             | Not applicable                                                         |
| with regard to ecotoxicological data          | Not applicable                                                         |

Analytical methods
(Regulation (EU) No 283/2013, Annex Part B, point 4 and Regulation (EU) No 284/2013, Annex Part B, point 5)

Analytical methods for the micro-organism
(Regulation (EU) No 283/2013, Annex Part A, point 4.1 and Regulation (EU) No 284/2013, Annex Part A, point 5.1)

| Category                                      | Details                                                                 |
|-----------------------------------------------|------------------------------------------------------------------------|
| Manufactured microorganism (principle of method) | Confidential information, see Volume 4.                                  |
| Impurities and contaminating microorganisms in manufactured material (principle of method) | Confidential information, see Volume 4.                                  |
| Microbial Pest Control Product (principle of method) | Confidential information, see Volume 4.                                  |

Analytical methods for residues (viable and non-viable) in exposed compartments and organisms
(Regulation (EU) No 283/2013, Annex Part A, point 4.2 and Regulation (EU) No 284/2013, Annex Part A, point 5.2)

| Category                                      | Details                                                                 |
|-----------------------------------------------|------------------------------------------------------------------------|
| Analysis of the active microorganism (principle of method) | Not applicable since no consumer exposure is expected.                  |
| Analysis of relevant metabolites (principle of method)   | Not applicable since no consumer exposure is expected.                  |
Effects on human health

(Regulation (EU) N° 283/2013, Annex Part A, point 5 and Regulation (EU) N° 284/2013, Annex Part A, point 7)

Medical data (including medical surveillance on manufacturing plant personnel) (MA 5.1.1)

The potential of Bti AM65-52 to cause food poisoning has not been fully elucidated. It has been shown that the strain is able to produce enterotoxins, indicating that it might have the ability to cause the diarrheal syndrome of *B. cereus* type food poisoning. However, no food poisoning case where Bti strain AM65-52 was the causative agent has been reported.

No adverse reactions in individuals as a result of contact with this microbial during its development, manufacture, preparation have been documented or reported up until 2016 (detailed documentation on the health surveillance programme is missing – data gap).

Sensitisation (MA 5.2.1 & MP 7.2.3)

Four studies are available that describe sensitisation observations following worker or bystander exposure to *Bacillus thuringiensis*, two of these ssp. *israelensis* strain AM65-52 (VectoBac formulations, equivalent to Gnatrol SC) and two ssp. *kurstaki*. The results indicate that production of specific IgE can occur in workers exposed to products containing Bti, but do not show any effects on the incidence or prevalence of respiratory symptoms, lung function or bronchial responsiveness.

Buehler test with Vectobac Technical Powder: positive

Due to the presence in the technical grade material of a sensitising non-active ingredient, classification is warranted.

The following sentences are required:

Contains *Bacillus thuringiensis* ssp. *israelensis* AM65-52. Micro-organisms may have the potential to provoke sensitising reactions.

EUH208 - Contains 1,2-benzisothiazol-3(2H)-one (BIT). May produce an allergic reaction.

Acute oral infectivity, toxicity and pathogenicity (MA 5.2.2.1 & MP 7.1.1)

1) Rat LD$_{50}$ > 5000 mg/kg bw, corresponding to 5.65 x 10$^{11}$ CFU/kg bw. No mortalities or significant toxic effects, clearance not investigated.

2) Rat LD$_{50}$ > 6.7 x 10$^7$ CFU/animal. No deaths or significant toxic/pathogenic effects, full clearance by day 22.

3) Rat LD$_{50}$ > 2.0 x 10$^9$ CFU/animal. No deaths or significant toxic/pathogenic effects, full clearance in males at day 21 but not in females (blood, liver, spleen, kidney and lungs).

Acute intratracheal/inhalation infectivity, toxicity and pathogenicity (MA 5.2.2.2 & MP 7.1.2)

1) Rat (intratracheal): LC$_{50}$ > 2.85 x 10$^7$ CFU/animal. No mortalities. Clinical signs: ruffled coat, lethargy, and effects on body posture, respiration and locomotor activity on days 1-2, likely a consequence of the administration. Clearance incomplete at 50 days (remaining in spleen, liver, caecum and lungs).

2) Rat (intratracheal) LC$_{50}$ > 8 x 10$^7$ CFU/animal. No deaths or significant toxic/pathogenic effects, incomplete clearance (supportive information)

3) Rat (whole body): LC50 > 2.84 mg/L. No mortalities, transient clinical signs, clearance not investigated.

4) Mouse (intratracheal): NOAEL for Vectobac 12AS: 10$^7$ CFU/mouse. Live vegetative cells caused hypoactivity, hunched-over appearance, ruffled fur and respiratory distress.

5) Mouse (intratracheal): viable cells of Bti can translocate to spleen and liver of immunocompetent animals in a dose-dependent manner. Unclear whether translocation was through lungs or GI tract (supportive information).

6) Mouse (intranasal): with 10$^8$ CFU/mouse *B. thuringiensis* serotype 14: 40% mortality. Large haemorrhagic liquefied
lesions of the lungs and liver. Lung histology: acute bronchitis associated with ulceration, injuries of the mucociliary apparatus, oedema and alveolar damage. Possible causative agent: haemolysin.

1) Rat i.v.: LD$_{50}$ > 10$^7$ CFU/rat. No adverse effects observed. Total clearance observed from brain, blood, and caecum contents, but not from lungs, spleen, liver, lymph nodes and kidney (day 50) (supportive).

2) Mouse i.p.: LD$_{50}$ > 10$^8$ CFU/mouse. No adverse effects observed. Clearance not investigated (supportive).

**In vitro studies: no data.**

Not required

Oral 90-day dog: NOAEL 5 x 10$^6$ spores/animal. No adverse effects observed.

Inhalation 14-day rat: NOAEC 1.18-1.84 x 10$^6$ spores/L. No adverse effects observed.

Inhalation 14-day mouse: LOAEL 4.2 x 10$^4$ 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.

**Rabbit: no acute dermal toxicity/infectivity; mild skin and eye irritation.**

**Technical Grade Active Ingredient (TGAI):** Combined mouse bone marrow micronucleus assay, rat bone marrow chromosomal aberration assay and mouse sperm morphology assay: with Bti SH14 but the strain was not specified, no evidence of the test substance reaching in the target cells in sufficient concentrations and only one dose group was used. For these reasons, the study is unacceptable.

Metabolites:

1) Low acute oral toxicity: delta-endotoxins are digested in the mammalian gastrointestinal tract and does not damage mammalian intestinal cell’s membrane integrity

2) Mouse in vivo micronucleus assay (i.p.):

- Spores crystal complexes of Btk genetically modified to express one single delta-endotoxin: Cry1Aa: positive, stat sign and dose-related increase in MN-PCE
- Cry1Ac: stat sign increase in MN-PCE at the only dose level tested

The result of the study was equivocal. Unclear whether toxins had been solubilised/activated prior to administration or not (data gap).

These Cry toxins are not produced by Bti AM65-52; however, corresponding data is lacking for Cry4Aa, Cry4Ba, Cry10A, Cry11A, Cyt1Aa, Cyt2Ba, and Cyt1Ca.

**No data**

**Genotoxicity – in vivo studies in germ cells (MA 5.5)**

**Reference values**

**AOEL**

As no exposure models exist for microbials, setting an AOEL would be of low relevance to the risk assessment.
For the potential risk after repeated exposure by inhalation, the use of respiratory protective equipment for the operators and workers might be considered.

| ADI |
| --- |
| The threshold of $10^5$ CFU/g food is applicable to cover the risk of food-borne poisonings caused by the B. cereus group of micro-organisms. |

| ARfD |
| --- |
| The threshold of $10^5$ CFU/g food is applicable to cover the risk of food-borne poisonings caused by the B. cereus group of micro-organisms. |

**Exposure**

| Exposure (operator, workers, bystander, resident) (MA 6.1 & MP 7.3, 8.0) |
| --- |
| In the absence of a quantitative risk assessment, the use of respiratory protective equipment for the operators and workers might be considered to reduce the exposure via inhalation. |

**Residues in or on treated products, food and feed**

(Regulation (EU) N° 283/2013, Annex Part B, point 6 and Regulation (EU) N° 284/2013, Annex Part B, point 8)

**Viable residues**

The intended use of *Bacillus thuringiensis* ssp *israelensis* (serotype H-14) strain AM65-52 is for the control of larvae of fungus gnats (*Sciaridae*) for the cultivation of non-edible ornamental plants. Application is done in protected vessels (pots) and no consumer exposure is envisaged, therefore a consumer risk-assessment is not considered necessary if the treated soil is not use for the cultivation of edible crops. In case that this restriction does not apply, a data gap was set for quantification of viable counts in soil after treatment to characterise and to conclude on the residue behaviour of *Bacillus thuringiensis* ssp. *israelensis* (serotype H-14) strain AM65-52 noting that edible crops may be grown in rotation on treated soil (see Section 3).

**Non-viable residues**

Not applicable
Fate and behaviour in the environment

(Regulation (EU) N° 283/2013, Annex Part B, point 7 and Regulation (EU) N° 284/2013, Annex Part B, point 9)

Persistence and multiplication (competitiveness) in soil, water and air

*Bacillus thuringiensis* subsp. *israelensis* and its crystalline proteins (δ-endotoxins) occur naturally in the environment.

Long-term studies indicate that even though recycling of Bti seems to be possible in larval carcasses, the long-term effects of field application on the abundance of Bti in the environment seem to be moderate.

Various studies on persistence of Bt crystalline proteins are available.

The agreed degradation endpoints for modelling purposes are:

- DT$_{50}$ soil = 41.3 d
- DT$_{50}$ 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.

| DT$_{50}$ Soil (days) | Experiment/Cry Protein                                      |
|----------------------|------------------------------------------------------------|
| 0.0208               | Free protoxin/toxin                                         |
| 0.1667               | Free protoxin/toxin                                         |
| 2.7                  | $^{14}$C labelled protoxins, sterilised, amended soil       |
| 5.2                  | $^{14}$C labelled protoxins, sterilised, amended soil       |
| 3                    | Natural soil                                               |
| 21                   | Natural soil                                               |
| 7                    | Natural soil (laboratory)                                   |
| 15                   | $^{14}$C[Cry1Ac Natural soil (laboratory)                   |
| 9.8                  | Cry1Ab and Cry1Ac Natural soil (laboratory)                 |
| 12.7                 | Cry1Ab and Cry1Ac Natural soil (laboratory)                 |
| 6.6 (calculated from DT$_{50}$) | Cry3Bb1 and Natural soil (laboratory)               |
| 12 (calculated from DT$_{50}$) | Cry3Bb1 and Natural soil (laboratory)               |
| 14                   | Cry1Aa Natural soil (laboratory)                            |
| 1.5                  | Cry1Ac Natural soil (laboratory)                            |
| 26.5                 | Cry1Ab paddy soil (aerobic laboratory)                      |
| 41.3                 | Cry1Ab paddy soil (aerobic laboratory)                      |
| 38.5                 | Cry1Ab paddy soil (aerobic laboratory)                      |
| 19.6                 | Cry1Ab paddy soil (aerobic laboratory)                      |
| 23.7                 | Cry1Ab paddy soil (aerobic laboratory)                      |
| 9                    | Cry1Ac Natural soil (laboratory)                            |
| 10                   | Cry1Ac Natural soil (laboratory)                            |
| 0.75                 | Cry1Ab Natural soil (laboratory)                            |
10.89       Cry1Ab Natural soil (laboratory)
1.8        Cry1Ab Natural soil (laboratory)
4          Cry1Ab Natural soil (laboratory)
17.75      Cry1Ac Natural soil (laboratory)
18.05      Cry1Ac Natural soil (laboratory)

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

The available studies indicate that *B. thuringiensis* ssp. *israelensis* spores are rather immobile after introduction into the environment.

The agreed mobility endpoints for modelling purposes are: $K_{doc} = 1000\text{mL/g}$

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 (H2O) | 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 $(n=25)$ | 16–707 (250)               | 0.59–243 (51)            | 4.3–8.6 (6.1) | 0–39 (10.5)     | 1–70 (23)       |
### Minimum and maximum values (average in brackets) of affinity ($K_a / \text{dm} \cdot \text{kg}^{-1}$) 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 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) |

**PEC soil**

| Microorganism | Spores and crystalline proteins |
|----------------|-------------------------------|
| Method of calculation | |
Application data

Crop: Ornamentals
Depth of soil layer: 5cm
Soil bulk density: 1.5g/cm³
% plant interception: no crop interception
Number of applications: 3
Interval (d): 3
Application rate(s): 3 x 12.30 kg MPCA/ha (1 x 10¹⁴ CFU/ha) and 3 x 1.675 kg cry protein/ha (based on a crystalline protein content of 13.6%)

PECsoil
(mg/kg; CFU/kg)
Initial
Spores:
49.2 mg/kg dw soil or
4 x 10⁸ CFU/kg dw soil
Crystalline proteins:
6.67 mg crystalline protein/kg dw soil

PEC groundwater

Microorganism
Satisfactory information was not provided in relation to potential interference of Bacillus thuringiensis ssp. israelensis strain AM65-52 with the analytical systems for the control of the quality of drinking water (data gap).

Crystalline protein:
Models used: FOCUS PEARL 4.4.4. and FOCUS PELMO 5.5.3
Soil DT₅₀ 42 days
Soil adsorption Kₐₐ₅ 1000 mL/g 1/n=0.9.

Application rate
3 x 1.675 kg cry protein/ha (based on a crystalline protein content of 13.6%) with spray interval of 3 days.

PEC(gw) - FOCUS modelling results (80th percentile annual average concentration at 1m)

| Scenario       | Parent (µg/L) |
|----------------|--------------|
| Chateaudun     | <0.001       |
| Hamburg        | <0.001       |
| Jokioinen      | <0.001       |
| Kremsmunster   | <0.001       |
| Porto          | <0.001       |
| Thiva          | <0.001       |

| Scenario       | Parent (µg/L) |
|----------------|--------------|
| Chateaudun     | <0.001       |
| Hamburg        | <0.001       |
| Jokioinen      | <0.001       |
| Kremsmunster   | <0.001       |
| Porto          | <0.001       |
| Thiva          | <0.001       |

PEC surface water

Microorganism
For the representative use by drench via a sprayer or irrigation on soil bound systems in permanent greenhouse the exposure to surface water is expected to be negligible.

Method of calculation

Application rate
Not applicable
Section 5  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 (MA 8.1 & MP 10.1)

| Dose (mg MPCA/kg bw) | Test material | Crop | Species | Time-scale | Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects) |
|----------------------|---------------|------|---------|------------|----------------------------------------------------------------------------------|
| 3077 mg a.i./kg/day for 5 days | VectoBac technical material | Not relevant | Mallard duck *Anas platyrhynchos* | 30 days | Occasional effects of wing droop and feather picking were observed. Necropsy revealed incidents of linear, flat, cream coloured plaques and multiple yellow plaques in the air sac. No effects were considered treatment related. |
| 3077 mg a.i./kg/day for 5 days | VectoBac technical material | Not relevant | Bobwhite quail *Colinus virginianus* | 30 days | Occasional effects of loss of coordination and toe and wing picking. Necropsy revealed incidents white material, either pasty or fluid in nature in the crop. No effects were considered treatment related. |

Effects on mammals

| Application rate (kg MPCA/ha) | Test material | Crop | Category (e.g. herbivorous mammal) and species | Time-scale | Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects) |
|-------------------------------|---------------|------|-----------------------------------------------|------------|----------------------------------------------------------------------------------|
| Studies on mammals are presented under section 2. |

Effects on other terrestrial vertebrates

| Application rate (kg MPCA/ha) | Test material | Crop | Category (e.g. amphibian) and species | Time-scale | Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects) |
|-------------------------------|---------------|------|-----------------------------------------------|------------|----------------------------------------------------------------------------------|
| No studies on other terrestrial vertebrates were available. |

Effects on aquatic organisms (MA 8.2 & MP 10.2)

| Group | Test material | Time-scale | Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects) |
|-------|---------------|------------|----------------------------------------------------------------------------------|
| **Laboratory tests** | | | |
| **Fish** | | | |
| *Oncorhynchus mykiss* (Rainbow trout) | Bti | 96-hour | 96 h LC50 >1.8 x 10¹⁰ CFU/L (nom) >370 mg MPCA/L (nom) |
| | | | 300 mg/L 0% mort 370 mg/L 10% mort in 1/3 replicates (single fish) |
| *Lepomis macrochirus* (Bluegill sunfish) | Bti | 96-hour | 96 h LC50 >2.9 x 10¹⁰ CFU/L >600 mg MPCA/L (nom) |
| | | | 300 mg/L 13% mort 600 mg/L 23% mort |
| Organism                                | Test Material          | Duration | NOEC/LOEC Results                                                                 |
|-----------------------------------------|------------------------|----------|----------------------------------------------------------------------------------|
| *Oncorhynchus mykiss* (Rainbow trout)   | Vectobac Technical     | 32-day   | No NOEC could be determined due to effects at all treatment levels. LOEC 1.1 x 10^10 CFU/L (mm) (0.055 g MPCA/L) + 1.72 x 10^9 CFU/g (mm) 1.1 x 10^10 CFU/L (mm) (0.055 g MPCA/L) + 1.72 x 10^9 CFU/g (mm) 8% ↓ length 25% ↓ bw |
| *Lepomis macrochirus* (Bluegill sunfish)| Vectobac Technical     | 32-day   | NOEC 1.2 x 10^10 CFU/L (mm) (0.06 g MPCA/L) + 1.31 x 10^9 CFU/g (mm)             |
| *Cyprinodon variegatus* (Sheepshead minnow)| Vectobac Technical | 32-day   | NOEC 1.3 x 10^10 CFU/L (mm) (0.065 g MPCA/L) + 2.1 x 10^9 CFU/g (mm) 1.3 x 10^8 CFU/L (mm) (0.065 g MPCA/L) + 2.1 x 10^9 CFU/g (mm) 17% mort |
| *Danio rerio*                           | Brazilian Bti strain   | 30-day   | No mortality, no visible adverse effects                                          |
| *Oreochromis niloticus*                 |                        |          | Increased frequency of necrotic cells (treatment considered invasive by authors) |
| **Invertebrates**                       |                        |          |                                                                                  |
| *Daphnia magna*                         | Vectobac Technical     | 10-day   | LC50 > 2.4 x 10^9 CFU/L >50 mg/L (nom)                                           |
| *Daphnia magna*                         | Vectobac Technical     | 21-day   | LOEC 1 x 10^8 CFU/L 1 x 10^8 CFU/L 19% repro (n.s.) 14% mort (n.s.) 9% growth (n.s.) 1 x 10^8 CFU/L 55% repro (n.s.) 14% mort (n.s.) 9% growth (n.s.) 1 x 10^10 CFU/L 92% repro 71% mort 53% growth |
| *Palaemonetes vulgaris* (Grass shrimp)  | Vectobac Technical     | 31-day   | NOEC = 2.0 x 10^10 CFU/g (mm diet conc) + 1.8 x 10^9 CFU/L (mm aq conc) |
| *Amphiascus minutus* (Harpacticoid copepod) | Vectobac Technical | 10-day   | NOEC = 50 mg/kg (nom). Endpoint was not reported in CFU/L.                      |
| *Daphnia sp.* Field microcosm           | Vectobac 12 AS         | 21-day   | LOEC 0.16 µL/L Significant changes in age structure at 0.16 and 0.50 µL/L in comparison with the control after 21 days. |
| *Daphnia sp.* Field microcosm           | Vectobac 12 AS         | 21-day   | NOEC 0.16 µL/L Significant effects in population density at 0.50 µL/L in comparison with the control after 21 days. |
| **Algae**                               |                        |          |                                                                                  |
| *Euglena spp.*, *Chlamydomonas sp.*, *Oedogonium sp* and mixed algal cultures | Purified toxins from Bti (not specified), unclear dose | 48h     | “not inhibitory”                                                                 |
| **Aquatic plants**                      |                        |          |                                                                                  |
| No studies on aquatic plants were available |                        |          |                                                                                  |
Effects on bees (MA 8.3 & MP 10.3)

| Species             | Test material                        | Crop            | Route and Time-scale | Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects) |
|---------------------|--------------------------------------|-----------------|----------------------|-----------------------------------------------------------------------------------------|
| **Laboratory tests**|                                      |                 |                      |                                                                                         |
| *Apis mellifera*    | VectoBac WG (Bti AM65-52)            | Not relevant; laboratory test. | Adult Oral 48h       | LD₅₀ > 108.04 μg a.i./bee Study has not been evaluated further since it is performed on an old product. |
| *Apis mellifera*    | VectoBac WG (Bti AM65-52)            | Not relevant; laboratory test. | Adult Contact 48h    | LD₅₀ > 100 μg a.i./bee Study has not been evaluated further since it is performed on an old product. |
| *Apis mellifera*    | VectoBac technical material (Bti AM65-52) | Not relevant; laboratory test. | Adult Oral 14-day    | NOEL = 2400 g/acre (highest tested dose)                                                   |
| *Apis mellifera*    | Vectobac 12 AS (Bti AM65-52)         | Not relevant; laboratory test. | Adult Oral 48h       | LD₅₀ > 115.47 μg a.i./bee (2% mortality)                                                  |
| *Apis mellifera*    | Vectobac 12 AS (Bti AM65-52)         | Not relevant; laboratory test. | Adult Contact 48h    | LD₅₀ > 100 μg a.i./bee (6% mortality)                                                     |
| *Apis mellifera*    | VectoBac technical material (Bti AM65-52) | Not relevant; laboratory test. | Larvae. Single exposure. 72h | LD₅₀ >100μg a.i./larva 2.5 μg a.i./larva: 8% mort (not sign) 50 μg a.i./larva: 11% mort (not sign) 100 μg a.i./larva: 19% mort (sign) |
| *Bombus terrestris* | Vectobac 12 AS                       | Not relevant; laboratory test. | Contact and oral. Duration unknown. | No details were provided. Classified as harmless (<25% effect) (mortality from contact exposure) Dose: Contact 50 μL Oral 0.06 g a.i./L.¹ |
| **Other types of test** |                                      |                 |                      |                                                                                         |
| *Apis mellifera*    | VectoBac WG                          | -               | Health of bee hives monitored during 1 year in five locations in France monitored following wetland treatments with Vectobac WG. | No adverse effects attributed to the treatment. |

¹Considered supportive only due to poor description of the study.

Effects on terrestrial arthropods other than bees (MA 8.4 & MP 10.4)

| Species               | Stage     | Test material                        | Dose (kg MPCA/ha) | Route and Time-scale | Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects) |
|-----------------------|-----------|--------------------------------------|-------------------|----------------------|-----------------------------------------------------------------------------------------|
| *Typhlodromus pyri*   | protonymphs | VectoBac TP (Bti AM65-52)            | 2.25 kg a.s./ha in 200L/ha water | 14 d Laboratory test glass plates (2D) | ER₉₀ > 2250 g/ha 0 % mortality 5.2 % inhib of reproduction (not sign) |
| *Aphidius rhopalosiphi* | adults   | VectoBac TP (Bti AM65-52)            | 2.25 kg a.s./ha in 200L/ha water | 14 d Laboratory test glass plates (2D) | ER₉₀ > 2250 g/ha 6.9 % mortality 2.4 % increase of reproduction (not sign) |
Anthonomus grandis

Bti strain T14001 and recombinant Bti strains expressing the individual Cyt1Aa, Cry4Aa, Cry4Ba and Cry11Aa toxins

Feeding tests

LC₅₀ for Bti T14001: 0.74 mg/ml. Individual toxins induced toxic effects but not enough for LC₅₀ calculation

Effects on other terrestrial invertebrates (MA 8.5 & MP 10.5)

Species

Toxicity, infectivity and pathogenicity:
(endpoint, value or other description of effects)

Further information

| Species          | NOEC = 1000 mg/kg food + 1000 mg/kg in the soil |
|------------------|-----------------------------------------------|
| Control          | 40% bw increase from day 0-30                 |
|                  | 100 mg/kg food + 1000 mg/kg in the soil       |
|                  | 49% bw increase from day 0-30                 |
|                  | 30-day Artificial soil; VectoBac TP (Bti AM65-52); 1000 mg/kg food at day 0 and day 14 |

Effects on soil microorganisms (MA 8.6 & MP 10.6)

One study was available evaluating the release of Bti (different strain than the active organism) in field soils in Sweden. This study reported that 7 weeks following the release, the fraction of Bt/cereus-like bacteria was similar to before the release. Furthermore, based on the information available in Vol. 3, MA, B8, it has been concluded that long-term effects of field Bti application on the abundance of Bti in the environment seem to be moderate.

Additional studies (MA 8.7 & MP 10.7)

No relevant additional studies.