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| REGISTRATION REPORT Part B  Section 5: Environmental Fate  Detailed summary of the risk assessment |
| Product code:  FORAY® 76B (ABG-6431)  Active Substance:  Bacillus thuringiensis subsp. kurstaki strain ABTS-351  206.5 g/L |
| Central Zone  **(zRMS: Poland)** |
| CORE ASSESSMENT |
| Applicant: XXXX  Submission Date: August 2023  Evaluation date: May 2024  **MS Finalisation date: September 2024** |

Version history

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| **When** | **What** |
| August 2023 | Initial version submitted by the applicant for Art. 43 |
| May 2024 | Version evaluated by zRMS PL |
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Table of Contents

[IIIM 9 FATE AND BEHAVIOUR IN THE ENVIRONMENT FOR THE MICROBIAL PEST CONTROL PRODUCT (RATIONALE TO WAIVE TESTING, BASED ON ADEQUACY OF INFORMATION PROVIDED FOR MPCA, TO PERMIT AN ASSESSMENT OF THE FATE AND BEHAVIOUR OF MPCP IN THE ENVIRONMENT 4](#_Toc142484647)

[IIIM 9.1 Fate and behaviour in soil 12](#_Toc142484648)

[IIIM 9.1.1 Persistence and multiplication in soil 12](#_Toc142484649)

[IIIM 9.1.2 Mobility in soil 13](#_Toc142484650)

[IIIM 9.1.3 Possible contamination with metabolite 14](#_Toc142484651)

[IIIM 9.1.4 Predicted environmental concentration in soil 14](#_Toc142484652)

[IIIM 9.2 Fate and behaviour in water 18](#_Toc142484653)

[IIIM 9.2.1 Persistence and multiplication in water 18](#_Toc142484654)

[IIIM 9.2.2 Predicted Environmental Concentrations in water (PECSW) for the Formulation, Active Substance and Crystal Proteins 26](#_Toc142484655)

[IIIM 9.3 Fate and behaviour in groundwater 32](#_Toc142484656)

[IIIM 9.3.1 Predicted Environmental Concentrations in Groundwater (PECGW) 32](#_Toc142484657)

[IIIM 9.4 Fate and behaviour in air 36](#_Toc142484658)

[Appendix 1: List of data submitted in support of the evaluation 37](#_Toc142484659)

[Appendix 2: GAP table 38](#_Toc142484660)

[Appendix 3: Additional Information 45](#_Toc142484661)

# IIIM 9 FATE AND BEHAVIOUR IN THE ENVIRONMENT FOR THE MICROBIAL PEST CONTROL PRODUCT (RATIONALE TO WAIVE TESTING, BASED ON ADEQUACY OF INFORMATION PROVIDED FOR MPCA, TO PERMIT AN ASSESSMENT OF THE FATE AND BEHAVIOUR OF MPCP IN THE ENVIRONMENT

This registration report is submitted to the Ministry of Agriculture and Rural Development (Poland) as zonal Rapporteur Member State (zRMS) and cMS (DE, HU, RO) in August 2023 to support the authorisation of the plant protection product (PPP) Foray® 76B (product code ABG-6431) in the EU Central Zone under Article 43 of Regulation (EC) No. 1107/2009. The formulation Foray® 76B is an aqueous suspension concentrate (SC) containing 206.5 g/L the active substance *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351. The content of *B. thuringiensis* subsp*. kurstaki* strain ABTS-351 in Foray® 76B range between 1.17 x 1013 CFU/L and 1.69 x 1013 CFU/L (nominal concentration of 1.51 x 1013 CFU/L). It is currently authorised across the EU for use as an insecticide to control lepidopteran defoliating caterpillars on deciduous and coniferous forest, pine trees, ornamental trees and shrubs or amenity areas (parks, gardens).

*B. thuringiensis* subsp. *kurstaki* strain ABTS-351 was first assessed for approval for use as PPP in the EU in 2008 by Denmark as Rapporteur Member State (RMS). It was included in Annex I of Directive 91/414/EC as a new active substance on 01 May 2009. Application for renewal of the active substance was submitted to Denmark (RMS) and the Netherlands (co-RMS) in 2016 under Regulation (EC) No. 1107/2009, replacing Directive 91/414/EC. EFSA Conclusion on the peer review of risk assessment of *B. thuringiensis* subsp. *kurstaki* strain ABTS-351 was published on 22 October 2021 (EFSA Journal 2021;19(10):6879). No critical areas of concern were identified in the EFSA Conclusion. Renewal of approval of *B. thuringiensis* subsp. *kurstaki* strain ABTS-351 was granted on 23 May 2023 (entry into force 1 July 2023); Commission Implementing Regulation (EU) 2023/999.

When the AIR 4 dossier was submitted for EU renewal of *B. thuringiensis* subsp*. kurstaki* strainABTS-351, an application to demonstrate technical equivalence of *B. thuringiensis* subsp*. kurstaki* strainABTS-351produced at a new manufacturing site for XXXX, was also submitted to Denmark. Technical equivalence was granted in January 2018.

DiPel® DF (product code ABG-6404) is the representative formulation used to support the application for renewal of approval of *B. thuringiensis* subsp. *kurstaki* strain ABTS-351, thus have been evaluated during the approval process. The representative uses are outdoor vegetables (cabbage) and indoor vegetables (tomato).

Relevant EU agreed endpoints and conclusions of the risk assessments drawn during the previous EU Renewal of *Btk* ABTS-351 are as follows (EFSA Journal 2021; 19(10):6879 and RAR, 2020):

| **Endpoint**  (EFSA Journal 2021;19(10):6879) | **Active substance** |
| --- | --- |
| **Persistence and multiplication** |  |
| In soil: | ***Bacillus thuringiensis***occurs naturally and ubiquitously in the environment. It is a common component of the soil microbiota and has been isolated from most terrestrial habitats.  Although the spores of *Bacillus thuringiensis* subsp*. kurstaki* strain ABTS-351 may be persistent (from days to years) in the soil under natural field conditions, there is a low potential for germination of spores in the bulk soil where nutrient levels are generally more limited and thus, a low potential for spore germination, growth and re-sporulation will minimise multiplication.  **Crystal proteins** (and or ẟ-endotoxins): a conservative degradation rate in soil was agreed: **DT50 soil 41.3 d** **at 20°C**  PECsoil calculations a,b) considering no crop interception:  ***Btk* ABTS-351:**  All yearly applications dosed as a single application  PEDsoil: 8.5 × 108 CFU/kg (corresponding to PECsoil of 10.7 mg product/kg and 5.76 mg a.s./kg)  **Crystal Protein:**  PECsoil: 0.475 mg CryP/kg (considering soil DT50 41.3 days) |
| In water: | Information on *Bacillus thuringiensis* subsp*. kurstaki* strain ABTS-351 was not available so EFSA concluded that there was a (non-critical) outstanding issue, for information on its proliferation in natural surface water systems (Section 9 EFSA Journal 2021; 19(10):6879). Following the EFSA review, the European Commission draft Renewal Report (October 2022) stated:  *“Bacillus thuringiensis* is not an aquatic bacterium, and since water is not its natural habitat, germination of spores (and therefore, multiplication and production of secondary metabolites) is not expected.”  PEC in surface water a,b):  ***Btk* ABTS-351**  All yearly applications dosed as a single application; Rautmann drift value of 6.26% (vegetables; height of crop ≥ 0.5 m; more than 7 applications); 300 L/m2 ditch.  PEDSW: 3.0 × 106 CFU/L (corresponding to PECSW of 20.2 µg MPCA/L and 2.4 µg CryP/L)  **Crystal protein and FOCUS Step 2**  DT50 in soil of 42 d at 20°C (correct value is maximum aerobic soil DT50 of 41.3 d); Koc 1000 mL/g (representative worst-case); DT50 in water, sediment and water/sediment system 1000 d (correct value is DT50 in water, sediment and water/sediment system is 28 d at 20°C).  PECSW: 19.77 µg CryP/L  PECSED: 195.25 mg CryP/kg dw sediment |
| In air: | *B. thuringiensis* is generally not persistent in air  Following release to air, spores are expected to rapidly lose viability due to solar radiation. Fate and transport via air after application are unlikely to play a role in environmental exposure to *B. thuringiensis* subsp. *kurstaki* including *Btk* ABTS-351 spores and endotoxins. |
| Mobility: | The mobility of *B. thuringiensis* subsp. *kurstaki* including *Btk* ABTS-351 and its spores can be considered limited.  Crystal Proteins:  Agreed endpoints based on available information on crystal proteins for all *B. thuringiensis* species.  **Koc 1000 mL/g (representative worst-case)**  PEC in groundwater b)  FOCUS PEARL 4.4.4.; crop scenarios cabbage and tomatoes; all default location scenarios; DT50 in soil 42 d (correct value is DT50 in soil of 41.3 d at 20°C); Koc 1000 mL/g (representative worst-case).  PECGW values all <0.001 µg/L. |

a) Representative formulated product: DiPel® DF (ABG-6404)

b) PEC calculations based on field uses of 8 applications of 1 kg/ha of formulated product per application (corresponding to 0.540 kg MPCA/ha; 8.0 × 1013 CFU/ha per application; 64.8 g crystal protein, 7 d interval)

This document reviews any new environmental fate studies/information and modelling for the product Foray® 76B. A full risk assessment according to Uniform Principles which demonstrates that the product is safe for the environment is provided. In cases where country specific assessments for some data requirements are provided, this document should be read in conjunction with the relevant addenda.

For the implementation of the uniform principles according to Part II of the Annex to Regulation (EU) No 546/2011, the conclusions of the review report on the *B. thuringiensis* subsp. *kurstaki* strain ABTS-351, and in particular Appendices I and II thereof, as finalised shall be taken into account. In this overall assessment, any relevant concerns in the review report have been addressed within the current submission.

Appendix 1 of this document contains the list of references included in this document for support of the evaluation.

Appendix 2 informs of where to find the complete GAP table of intended uses for Foray® 76B.

Appendix 3 of this document contains any additional information used for the exposure assessments and to address the environmental fate and behaviour of *B. thuringiensis* subsp. *kurstaki* strain ABTS-351 in the environment.

Information on the detailed composition of Foray® 76B can be found in the confidential dossier of this submission (Registration Report - Part C).

**Table 9-1** **Critical use pattern of Foray® 76B**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** |
| **Use-No.** | **Member state(s)** | **Crop and/  or situation    (crop destination / purpose of crop)** | **F, Fn, Fpn  G, Gn, Gpn  or  I** | **Pests or Group of pests controlled    (additionally: developmental stages of the pest or pest group)** | **Application** | | | | **Application rate** | | | **PHI  (days)** | **Remarks:     e.g. g safener/synergist per ha** |
| Method / Kind | Timing / Growth stage of crop & season | Max. number  a) per use  b) per crop/ season | Min. interval between applications (days) | kg or L product / ha  a) min / max. rate per appl.  b) max. total rate per crop/season | g or kg as/ha   a) min / max. rate per appl.  b) max. total rate per crop/season | Water L/ha    min / max |
| 5 | PL | Pine trees | F | *Lymantria monacha* - LYMAMO  *Dendrolimus pini* - DENDPI | Spray | When caterpillars are visible following egg hatch & foliage growth sufficient for deposition | a) 1 - 4  b) 4 | 5 days | a) 2.5 L/ha  b) 10 L/ha | a) 0.52 kg a.s/ha  b) 2.06 kg a.s./ha | - | - | Application rate in CFU:  a) 3.77 x 1013 CFU/ha  b) 1.51 x 1014 CFU/ha |
| 6 | PL | Deciduous forest | F | *Operophtera brumata* - CHEIBR  *Tortrix viridana* - TORTVI | Spray | When caterpillars are visible following egg hatch & foliage growth sufficient for deposition | a) 1 - 4  b) 4 | 5 days | a) 2.5 L/ha  b) 10 L/ha | a) 0.52 kg a.s/ha  b) 2.06 kg a.s./ha | UVL application: 0-10 L/ha,  application of high pressure (10 bar): 200 L/ha,  application of low pressure (2-3 bar): 600 L/ha. | - | Application rate in CFU:  a) 3.77 x 1013 CFU/ha  b) 1.51 x 1014 CFU/ha |
| 7 | PL | Deciduous forest | F | *Euproctis chrysorrhoea* - EUPRCH | Spray | When caterpillars are visible following egg hatch & foliage growth sufficient for deposition | a) 1 - 2  b) 2 | 14 days | a) 3 L/ha  b) 6 L/ha | a) 0.619 kg a.s/ha  b) 1.24 kg a.s./ha | UVL application: 0-10 L/ha,  application of high pressure (10 bar): 200 L/ha,  application of low pressure (2-3 bar): 600 L/ha. | - | Application rate in CFU:  a) 4.53 x 1013 CFU/ha  b) 9.06 x 1013 CFU/ha |

| **1** | **2** | | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Use-No.** | | **Member state(s)** | **Crop and/  or situation    (crop destination / purpose of crop)** | **F, Fn, Fpn  G, Gn, Gpn  or  I** | **Pests or Group of pests controlled    (additionally: developmental stages of the pest or pest group)** | **Application** | | | | **Application rate** | | | **PHI  (days)** | **Remarks:     e.g. g safener/synergist per ha** |
| Method / Kind | Timing / Growth stage of crop & season | Max. number  a) per use  b) per crop/ season | Min. interval between applications (days) | kg or L product / ha  a) min / max. rate per appl.  b) max. total rate per crop/season | g or kg as/ha   a) min / max. rate per appl.  b) max. total rate per crop/season | Water L/ha    min / max |
| 8 | | RO | Coniferous forest | F | Lepidoptera caterpillars L1 to L4  *Choristoneura spp.* - CHONSP  *Lymantria monacha* - LYMAMO  *Thaumetopoea pityocampa* - THAUPI  *Dendrolimus pini* - DENDPI | Spray | When caterpillars are visible following egg hatch & foliage growth sufficient for deposition | a) 4 b) 4 | 5 days | a) 2 - 2.5 L/ha  b) 10 L/ha | a) 0.413 - 0.516 kg a.s/ha b) 2.06 kg a.s./ha | 0-10 L/ha (undiluted for ULV application) | - | Application rate in CFU:  a) 3.02 - 3.77 x 1013 CFU/ha  b) 1.51 x 1014 CFU/ha |
| 9 | | RO | Deciduous forest | F | Lepidoptera caterpillars L1 to L4  *Hyphantria cunea* - HYPHCU  *Malacosoma neustria* - MALANE  *Stilpnotia salicis* - LEUOSA  *Euproctis chrysorrhoea* - EUPRCH  *Lymantria dispar* - LYMADI  *Thaumetopoea processionea* - THAUPR  *Tortrix viridana* - TORTVI  *Operophtera bru*mata - CHEIBR | Spray | When caterpillars are visible following egg hatch & foliage growth sufficient for deposition | a) 4 b) 4 | 5 days | a) 2 - 2.5 L/ha  b) 10 L/ha | a) 0.413 - 0.516 kg a.s/ha b) 2.06 kg a.s./ha | 0-600 L/ha (undiluted for ULV application; high pressure application: 200L/ha and low pressure application 600 L/ha) | - | Application rate in CFU:  a) 3.02 - 3.77 x 1013 CFU/ha  b) 1.51 x 1014 CFU/ha |

| **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Use-No.** | **Member state(s)** | **Crop and/  or situation    (crop destination / purpose of crop)** | **F, Fn, Fpn  G, Gn, Gpn  or  I** | **Pests or Group of pests controlled    (additionally: developmental stages of the pest or pest group)** | **Application** | | | | **Application rate** | | | **PHI  (days)** | **Remarks:     e.g. g safener/synergist per ha** |
| Method / Kind | Timing / Growth stage of crop & season | Max. number  a) per use  b) per crop/ season | Min. interval between applications (days) | kg or L product / ha  a) min / max. rate per appl.  b) max. total rate per crop/season | g or kg as/ha   a) min / max. rate per appl.  b) max. total rate per crop/season | Water L/ha    min / max |
| 10 | HU | Deciduous forest species  (also on public areas) | F | Lepidopteran foliage pests  *Lymantria dispar* - LYMADI  *Hyphantria cunea* - HYPHCU  *Euproctis chrysorrhoea* - EUPRCH  *Aporia crataegi* - APORCR  *Thaumetopoea processionea* - THAUPR  *Tortrix viridana* - TORTVI  *Geometridae -* 1GEOMF  *Tortricidae* - 1TORTF  *Gracillariidae* - 1GRACF | Spray | When caterpillars are visible following egg hatch & foliage growth sufficient for deposition | a) 4 b) 4 | 5 days | a) 2 - 2.5 L/ha  b) 10 L/ha | a) 0.413 - 0.516 kg a.s/ha b) 2.06 kg a.s./ha | Ground spray: 600 - 1500 L/ha  Aerial spray: 60 - 80 L/ha | - | Application rate in CFU:  a) 3.02 - 3.77 x 1013 CFU/ha  b) 1.51 x 1014 CFU/ha |
| 11 | HU | Pine species  (also on public areas) | F | Lepidopteran foliage pests  *Dendrolimus pini -* DENDPI *Rhyacionia buoliana* - EVETBU *Gracillariidae* - 1GRACF | Spray | When caterpillars are  visible following egg hatch & foliage growth sufficient for deposition | a) 4 b) 4 | 5 days | a) 2 - 2.5 L/ha b) 10 L/ha | a) 0.413 - 0.516 kg a.s/ha b) 2.06 kg a.s./ha | Ground spray: 600 - 1500 L/ha  Aerial spray: 60 - 80 L/ha | - | Application rate in CFU:  a) 3.02 - 3.77 x 1013 CFU/ha  b) 1.51 x 1014 CFU/ha |

| **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Use-No.** | **Member state(s)** | **Crop and/  or situation    (crop destination / purpose of crop)** | **F, Fn, Fpn  G, Gn, Gpn  or  I** | **Pests or Group of pests controlled    (additionally: developmental stages of the pest or pest group)** | **Application** | | | | **Application rate** | | | **PHI  (days)** | **Remarks:     e.g. g safener/synergist per ha** |
| Method / Kind | Timing / Growth stage of crop & season | Max. number  a) per use  b) per crop/ season | Min. interval between applications (days) | kg or L product / ha  a) min / max. rate per appl.  b) max. total rate per crop/season | g or kg as/ha   a) min / max. rate per appl.  b) max. total rate per crop/season | Water L/ha    min / max |
| 12 | HU | Ornamental trees, bushes  (also on public areas) | F | Lepidopteran foliage pests  *Lymantria dispar* - LYMADI *Hyphantria cunea* - HYPHCU *Euproctis chrysorrhoea* - EUPRCH *Aporia crataegi* - APORCR *Thaumetopoea processionea* - THAUPR *Tortrix viridana* - TORTVI *Geometridae* - 1GEOMF *Tortricidae*  - 1TORTF *Gracillariidae -* 1GRACF *Dendrolimus pini* - DENDPI *Rhyacionia buoliana -* EVETBU | Spray | When caterpillars are  visible following egg hatch & foliage growth sufficient for deposition | a) 4 b) 4 | 5 days | a) 2 - 2.5 L/ha b) 10 L/ha | a) 0.413 - 0.516 kg a.s/ha b) 2.06 kg a.s./ha | Ground spray: 600 - 1200 L/ha  Aerial spray: 60 - 80 L/ha | - | Application rate in CFU:  a) 3.02 - 3.77 x 1013 CFU/ha  b) 1.51 x 1014 CFU/ha |
| 13 | DE | Coniferous forest, Deciduous forest | F | Lepidoptera caterpillars  L1 to L3 | Ground spray | When caterpillars are visible following egg hatch & foliage growth sufficient for deposition | a) 2  b) 2 | 14 days | a) 2 - 2.5 L/ha  b) 5 L/ha | a) 0.413 - 0.516 kg a.s/ha  b) 1.03 kg a.s./ha | 600 L/ha | - | Application rate in CFU:  a) 3.02 - 3.77 x 1013 CFU/ha  b) 1.51 x 1014 CFU/ha |
| 14 | DE | Coniferous forest, Deciduous forest | F | Lepidoptera caterpillars  L1 to L3 | Aerial spray | When caterpillars are visible following egg hatch & foliage growth sufficient for deposition | a) 2  b) 2 | 30 days | a) 2 - 2.5 L/ha  b) 5 L/ha | a) 0.516 kg a.s/ha  b) 1.03 kg a.s./ha | 70 L/ha | - | Application rate in CFU:  a) 3.77 x 1013 CFU/ha  b) 1.51 x 1014 CFU/ha |
| 15 | DE | Ornamental trees | F | Lepidoptera caterpillars  L1 to L3 | Ground spray | When caterpillars are visible following egg hatch & foliage growth sufficient for deposition | a) 1  b) 1 | NA | a) 2 - 2.5 L/ha  b) 5 L/ha | a) 0.413 - 0.516 kg a.s/ha  b) 0.516 kg a.s/ha | 600 L/ha | - | Application rate in CFU:  a) 3.02 - 3.77 x 1013 CFU/ha  b) 3.77 x 1013 CFU/ha |

|  |  |  |  |
| --- | --- | --- | --- |
| **Remarks**  **columns:** | 1 Numeration necessary to allow references  2 Use official codes/nomenclatures of EU Member States  3 For crops, the EU and Codex classifications (both) should be used; when relevant, the use situation should be described (e.g. fumigation of a structure)  4 F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application  5 Scientific names and EPPO-Codes of target pests/diseases/ weeds or, when relevant, the common names of the pest groups (e.g. biting and sucking insects, soil born insects, foliar fungi, weeds) and the developmental stages of the pests and pest groups at the moment of application must be named.  6 Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench  Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated. |  | 7 Growth stage at first and last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 38263-3152-4), including where relevant, information on season at time of application  8 The maximum number of application possible under practical conditions of use must be provided.  9 Minimum interval (in days) between applications of the same product  10 For specific uses other specifications might be possible, e.g.: g/m³ in case of fumigation of empty rooms. See also EPPO-Guideline PP 1/239 Dose expression for plant protection products.  11 The dimension (g, kg) must be clearly specified. (Maximum) dose of a.s. per treatment (usually g, kg or L product / ha).  12 If water volume range depends on application equipments (e.g. ULVA or LVA) it should be mentioned under “application: method/kind”.  13 PHI - minimum pre-harvest interval  14 Remarks may include: Extent of use/economic importance/restrictions |

The content of *B. thuringiensis* subsp*. kurstaki* strain ABTS-351 in Foray® 76B range between 1.17 x 1013 and 1.69 x 1013 CFU/L (nominal: 1.51 x 1013 CFU/L). As a worst-case, environmental exposure calculations have considered the maximum amount of 1.69 x 1013 CFU/L which equates to an individual application of 5.07 x 1013 CFU/ha when 3.0 L product/ha is applied.

For the full table of GAP uses, please refer to Appendix 2.

The impact of formulants is limited to short-term effects such as formation of stable spray dispersions or to facilitate uptake by target organisms, while their influence on long-term processes, such as degradation and distribution is negligible. Therefore, for the purposes of this risk assessment it is assumed that formulants do not influence the fate and behaviour of an active substance in the environment and are not considered further.

*Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 (*Btk*) is a naturally occurring, indigenous wild-type bacterium. As detailed in EFSA Journal 2021;19(10):6879, *Bacillus thuringiensis* subsp. *kurstaki* has the capability to form crystals of proteinaceous insecticidal δ-endotoxins (e.g. Cry1Aa, Cry1Ab, Cry1Ac, Cry1Ia, Cry2Aa and Cry2Ab; also referred to as crystalline proteins, cry proteins, insecticidal crystal proteins (ICPs), crystal proteins, parasporal crystals, parasporal protein-crystal, parasporal crystalline inclusions). These crystal proteins are responsible for the insecticidal mode of action of *Btk* and are a key component of the formulated product. It may also be that vegetative cells of *B. thuringiensis* subsp. *kurstaki* strain ABTS-351 can produce Vip (vegetative insecticidal proteins) and Sip (secreted insecticidal proteins) and Sip cytolytic proteins. Crystal proteins are present both inside and outside the spores, however, it is unknown as to what extent the crystal proteins (or vegetative cells) will be produced by *Btk* following application. Therefore, it was decided during the EU evaluation of *B. thuringiensis* subsp. *kurstaki* strain ABTS-351, (following the Pesticides Peer Review Meeting Teleconference 25; March 2020), that exposure assessments (*e.g.,* in surface water and groundwater) should be based upon the content of crystal proteins within the formulated product. It was also agreed at the expert meeting that it would be appropriate to read across degradation and adsorption end points from available datasets that contain endpoints for the different ẟ-endotoxins and/or crystal proteins. However, as these endpoints were not available for all ẟ-endotoxins present in *B. thuringiensis* subsp. *kurstaki* strain ABTS-351*,* it was agreed that the following most conservative values should be used in exposure calculations: a DT50 in soil value of 41.3 days; a Koc value of 1000 mL/g; and a DT50 in the water system of 28 days. EFSA considers that this information is also likely to cover the Vip and Sip proteins if produced. As detailed in EFSA Journal 2021;19(10):6879, these end-points are based on data taken from the Renewal Assessment Report of *Bacillus thuringiensis* subsp. *aizawai* strain GC-91 Volume 3 MA-B8.

Content of the Crystal Proteins and the Microorganism (MPCA) in the Formulated Product (Foray® 76B SC)

The technical powder (containing the active substance) used to formulate the product contains a maximum crystalline protein content of 12.7% (w/w) of the MPCA. Therefore, as a worst-case, this maximum has been used in exposure calculations.

As a worst-case, environmental exposure calculations for the Predicted Environmental Density (PED) have considered the maximum amount of 1.69 x 1013 CFU/L which equates to an individual application of 5.07 × 1013 CFU/ha when 3.0 L formulated product is applied. It is these maximum application rates in CFU/ha that have been used in environmental exposure calculations to cover the risk envelope for all uses.

## IIIM 9.1 Fate and behaviour in soil

### IIIM 9.1.1 Persistence and multiplication in soil

During the EU evaluation of *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 information was derived from published literature on different strains of *Bacillus thuringiensis* in relation to its persistence and multiplication in soil. Whilst there was no specific information available on the strain ABTS-351, studies on *Bacillus thuringiensis* subsp. *kurstaki* demonstrated that its spores can remain viable for many years and the species has been reported to have spores that can germinate in the rhizosphere of some plants. However, the weight of evidence suggests that spore germination does not occur in bulk soil with limited nutrient levels. The repeated use of *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 is expected to lead to an accumulation of spores in the soil environment, but multiplication in bulk soil is unlikely. As a result, it was concluded that the information is considered sufficient to address the uniform principles criterion and predicted environmental concentrations for soil have been calculated for the intended uses.

With regard to persistence of the crystalline proteins in soil, a range of data were evaluated for a number of *Bacillus thuringiensis* subspecies and the degradation of the crystalline proteins in soil under a range of conditions. It was concluded that persistence could be influenced by biotic (degradation by microorganisms) and abiotic factors (e.g., interactions with soil components such as adsorption or pH of the soil). The available rates of degradation are summarised in Table 9.1.1-1, which contains degradation data taken from the Renewal Assessment Report of *Bacillus thuringiensis* subsp. *aizawai* strain GC-91 (Volume 3 MA-B8). Overall, it was concluded that the crystal proteins/endotoxins do not persist or accumulate in soil. The longest DT50 value was agreed as the endpoint (a DT50 in soil of 41.3 days).

Table 9.1.1-1 DT50 properties of the crystal proteins in soil

|  |  |
| --- | --- |
| **Experiment/Cry Protein** | **DT50 Soil (days)** |
| Free protoxin/toxin | 0.0208 |
| Free protoxin/toxin | 0.1667 |
| 14C labelled protoxins, sterilised, amended soil | 2.7 |
| 14C labelled protoxins, sterilised, amended soil | 5.2 |
| Natural soil | 3 |
| Natural soil | 21 |
| Natural soil (laboratory) | 7 |
| [14C]Cry1Ac Natural soil (laboratory) | 15 |
| Cry1Ab and Cry1Ac Natural soil (laboratory) | 9.8 |
| Cry1Ab and Cry1Ac Natural soil (laboratory) | 12.7 |
| Cry3Bb1 and Natural soil (laboratory) | 6.6 (calculated from DT90) |
| Cry3Bb1 and Natural soil (laboratory) | 12 (calculated from DT90) |
| Cry1Aa Natural soil (laboratory) | 14 |
| Cry1Ac Natural soil (laboratory) | 1.5 |
| Cry1Ab paddy soil (aerobic 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 |
| Cry1Ac Natural soil (laboratory) | 9 |
| Cry1Ac Natural soil (laboratory) | 10 |
| Cry1Ab Natural soil (laboratory) | 0.75 |
| Cry1Ab Natural soil (laboratory) | 10.89 |
| Cry1Ab Natural soil (laboratory) | 1.8 |
| Cry1Ab Natural soil (laboratory) | 4 |
| Cry1Ac Natural soil (laboratory) | 17.75 |
| Cry1Ac Natural soil (laboratory) | 18.05 |
| **Endpoint** | **41.3** |

### IIIM 9.1.2 Mobility in soil

The potential mobility of *Bacillus thuringiensis* is low, and studies have shown that application of products containing *Bacillus thuringiensis* showed little or no movement of the microorganism. For further information please see the groundwater section below. As dispersal in soil is limited and there is an extremely low potential for groundwater contamination, no further assessment of the mobility of *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 is considered necessary.

Crystal proteins - mobility in soil

During the EU evaluation of *Bt* subsp. *kurstaki* strain ABTS-351, the adsorption data for the crystal proteins (Cry proteins) was taken from the RAR of *Bacillus thuringiensis* subsp. *aizawai* strain GC-91. The minimum and maximum values with the average in brackets of affinity (Kd / dm3 kg-1) for each of the crystal proteins in the soil samples are detailed below (as taken from EFSA, 2021). However, it was noted there is a different behaviour between the protoxins (ẟ-endotoxin crystals) and the toxins (soluble individual crystal proteins). Due to the differences between the two and because individual adsorption values for the three Cry toxins were not available (only the range and average) along with the organic carbon information of the soils, it was acknowledged that there is uncertainty in the underlying data. Therefore, it was agreed that a KOC of 1000 mL/g and 1/n of 1 would be used for exposure assessments as a representative worst-case derived from consideration of the available data.

Table 9.1.2-1 Minimum and maximum values (along with the average in brackets) of affinity (Kd/dm3 kg-1) for each of the crystal proteins in the soil samples

|  |  |  |  |
| --- | --- | --- | --- |
| **Protein** | **Cr1Ac** | **Cry2A** | **Cry1C** |
| Full sample set (*n*=41) | 1630–38400 (12100) | - | - |
| Soils under cereal culture (*n*=16) | 1630–28600 (10100) | - | - |
| Soils under (semi) natural land use (*n*=25) | 2820–38400 (13200) | - | - |
| Soils studied for all proteins (*n*=19) | 1630–24400 (11300) | 1560–29300 (16100) | 837–54600 (18300) |
| Soils under cereal culture (*n*=7) | - | 1550–26700 (4700) | 5000–54600 (19150) |
| Soils under (semi) natural land use (*n*=12) | - | 1560–29300 (13700) | 837–42900 (17700) |

### IIIM 9.1.3 Possible contamination with metabolite

The environmental fate and behaviour of the crystal proteins associated with *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 are discussed alongside the microorganism in each section. Please refer to each section for further details.

### IIIM 9.1.4 Predicted environmental concentration in soil

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| zRMS  Comments: | Calculation of PECS for active substance and formulation was submitted and accepted.  The endpoints used for PECs assessment were agreed at the EU level.   |  |  |  |  |  |  | | --- | --- | --- | --- | --- | --- | | **Use** | **Maximum total seasonal application rate [g MPCA/ha]** | **Formulated product**  **PECsoil [mg/kg]** | ***Btk* strain ABTS-351**  **PECsoil [mg/kg]** | ***Btk* strain**  **ABTS-351**  **PEDsoil [CFU/kg]** | **Cry Protein**  **PECsoil [mg/kg]** | | All Uses | 2080 \*\*\* | 4.48\*\* | 2.77 | 2.26 × 108 | 0.31\* (0.35) |   These values will be used in further risk assessment. |

Foray® 76B containing *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 is intended for use as a spray for the control of Lepidopteran pests on deciduous and coniferous forest, pine trees, ornamental trees and shrubs or amenity areas (parks, gardens). The Foray® 76B product will be applied up to four times per season, when caterpillars are visible and foliage growth is sufficient for egg deposition. The maximum recommended individual application rate is 2.5 L product per hectare, which is equivalent to 0.516 kg of *Btk* per hectare (rounded up to 0.520 kg/ha for calculations). The Predicted Environmental Density in soil (PEDsoil) of the microorganism, *Btk* strain ABTS-351, as well as the Predicted Environmental Concentrations in soil (PECsoil) of the MPCA (microbial pest control agent), formulated product and the associated crystal protein have been calculated. Calculations are based on the critical GAP. The agronomic input parameters are detailed in Table 9.1.4-1.

PED/PECsoil calculations have been performed considering a soil mixing depth of 5 cm and a soil density of 1.5 g/cm3. No crop interception was taken into account. For PED and PECsoil of *Btk*, no reduction in the microorganism between applications has been assumed (worst-case) and as such a seasonal total dose application has been input as a single application. For the crystal protein PECsoil values, calculations have been performed considering degradation between each application (using the minimum application interval specified in the GAP) and a degradation rate (DT50) of 41.3 days; see Table 9.1.4-2.

PECsoil for the formulated product has been calculated for a single application considering that almost immediately after application, it will break down to its constituent parts.

For PEDsoil calculations, the maximum content of 1.69 × 1013 CFU/L of the microorganism in the formulated product Foray® 76B was used as specified in Part C of this dossier (thus, 2.5 L product/ha is equivalent to 4.23 × 1013 CFU/ha and 3.0 L product/ha is equivalent to 5.07 × 1013 CFU/ha). For PECsoil calculations for the crystalline proteins, a maximum concentration of 12.7% (w/w) of the MPCA (active substance) has been used. The maximum GAP is for four applications of Foray® 76B at 2.5 L formulated product/ha with a minimum interval of 5 days which will cover the risk envelope for all GAPs for calculations for the active substance and the crystalline proteins. PECsoil values have also been calculated for the formulated product based on a single application as it is considered that upon application, the formulated product will breakdown to its constituent parts. An application of 3 L formulated product/ha is the highest individual dose specified in the GAP for applications to deciduous forest (2 x 619 g a.s./ha; 14-day interval) and therefore, for single applications, this maximum individual rate of application has been used.

Applications will be to deciduous and coniferous forest, pine trees, ornamental trees and shrubs or amenity areas (parks, gardens) and therefore, there will be significant foliar interception. OECD guidance No. 67[[1]](#footnote-2) states that crop interception can be included in calculations. However, during the EU evaluation of *Btk*, no crop interception was considered and so has not been considered for the calculations presented below; therefore, PED/PEC values represent a very worst-case. EFSA (2014)[[2]](#footnote-3) allows a minimum crop interception for vines and tree crops with foliage of 50% (first leaves; bush-berries and vines) and for tree crops ≥65% crop interception is appropriate for a full canopy (citrus and pome/stone fruit). These crop interception values show that there will be foliar interception for all uses within the GAP and so the calculations presented below are highly conservative.

Table 9.1.4-1 Agronomic input parameters for PECsoil calculations

|  |  |
| --- | --- |
| Use No. | All Uses |
| Crop | Pine trees, deciduous/coniferous forest, shrubs, ornamental trees and plants |
| Application rate  Formulated product [g/ha] \* | 4 × 2800 g Foray® 76B/ha  (1 x 3360 g Foray® 76B/ha\*\*) |
| Application rate  Active substance [g/ha] | 4 × 520 g MPCA/ha |
| Application rate  Active substance B*tk* [CFU/ha] | 4 × 4.23×1013 CFU/ha |
| Application rate  Crystal Proteins [g/ha] | 4 × 66.04 g CryP/ha |
| Number of applications/interval | 4/5 |
| Total Annual Application dose Active substance [g/ha] | 1 × 2080 g MPCA/ha |
| Total Annual Application rate  Crystal Proteins [g/ha] | 1 × 264.16 g CryP/ha |
| Total Annual Application dose *Btk* [CFU/ha] | 1 × 1.69×1014 CFU/ha |
| Crop interception [%] | 0% |
| Depth of soil layer [cm] | 5 cm for single season PED/PEC calculations. No accumulation expected to occur. |

\*The density of Foray® 76B is 1.12 g/cm3 for conversion of 2.5 or 3.0 L/ha product into g/ha; \*\*PEC calculations for the formulated product are based on single applications as it is considered that upon application, the formulation will break up into its constituent parts; therefore, the highest individual application rate has been used.

**Table 9.1.4-2** **Agreed EU Endpoint for the Crystal Proteins (EFSA, 2021**)

|  |  |
| --- | --- |
| **Endpoint** | **Active Substance Cry Protein** |
| DT50 (longest) | 41.3 days |

*PECsoil Calculation (total amount of applications per year)*

The process for calculating the PED/PECsoil values considering only the total amount per crop relies on a worst-case scenario. The initial PED/PECsoil is determined by using the application rate in g/ha and the total number of applications per year. In this scenario, all applications are applied at once without any consideration for degradation, growth, or crop interception.

PECsoil = (A × CF × n) ÷ (10000 × d × ρ)

PEDsoil = (A × n) ÷ (10000 × d × ρ)

PED: Predicted Environmental Density in CFU/kg soildw

PEC: Predicted Environmental Concentration in mg/kg soildw

- A is the application rate (g a.s./ha or CFU/ha).

- CF as Conversion factor 1 g = 1000 mg

- n is the number of applications per year

- 10000 is the conversion factor from ha to m2

- d is the thickness of the soil layer (default of 0.05 m)

- ρ is the density of soil (default of 1500 kg dry soil /m3)

The resulting PEC and PED values in soil are presented in Table 9.1.4-3.

**Table 9.1.4-3 PECsoil values following applications to crops**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Use** | **Maximum total seasonal application rate [g MPCA/ha]** | **Formulated product**  **PECsoil [mg/kg]** | ***Btk* strain ABTS-351**  **PECsoil [mg/kg]** | ***Btk* strain**  **ABTS-351**  **PEDsoil [CFU/kg]** | **Cry Protein**  **PECsoil [mg/kg]** |
| All Uses | 2080 \*\*\* | 4.48\*\* | 2.77 | 2.26 × 108 | 0.31\* (0.35) |

\* The PECsoil value for the crystal protein taking into account the degradation of DT50 of 41.3 days. PEC calculated with the GB PECsoil Calculator v1.0 ([UK HSE, 2023](https://www.hse.gov.uk/pesticides/pesticides-registration/data-requirements-handbook/fate/environmental-fate-models.htm)). Calculations in brackets along with the active substance PEC and PED calculations consider no degradation or reduction between applications and therefore, one application of the total seasonal dose;

\*\* Considers a maximum individual application of the formulated product of 1 x 3.0 L/ha (or 3360 g/ha considering a product density of 1.12 g/cm3) as it is assumed that soon after application the product will break down into its constituent parts.

\*\*\*Application rate of 516 g MPCA/ha rounded up to the nearest 10 g /ha and thus, the maximum total seasonal application is slightly greater than specified by the GAP table (4 x 520 g MPCA/ha). Where appropriate, calculations have been based on this total seasonal dose.

## IIIM 9.2 Fate and behaviour in water

### IIIM 9.2.1 Persistence and multiplication in water

During the EU evaluation, information on the persistence and multiplication of *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 in surface water was not available. Information on *Bacillus thuringiensis* subsp. *kurstaki* showed a decline in CFU levels in flowing water after applications were made, however, the authors attributed this to the dilution and removal effect by the flowing water. The available literature suggests that *Bacillus thuringiensis* is present in surface water and that it is likely that the species is capable of growing in freshwater environments under nutrient/oxygen-rich conditions. Overall, EFSA concluded that the information available on the persistence/multiplication/germination of the strain in natural surface water was insufficient to demonstrate that *Bacillus thuringiensis* subsp. *kurstaki* strain *ABTS-351* is likely to decline in surface water and identified a non-critical outstanding issue (See Section 9, EFSA Journal 2021; 19(10):6879). The zRMS disagreed with this along with the European Commission who in the draft renewal report for the active substance (October 2022) detailed:

“In surface water, spore densities are expected to be low, as EFSA identified high margins of safety for aquatic organisms when comparing the endpoints with expected spore concentrations after entry into surface water from the intended field uses. Likewise, as regards other secondary metabolites, *Bacillus thuringiensis* subsp. *kurstaki* ABTS-351 is applied as spores, which do not produce metabolites, unless germinating.

*Bacillus thuringiensis* is not an aquatic bacterium, and since water is not its natural habitat, germination of spores (and therefore multiplication and production of secondary metabolites) is not expected”.

Therefore, as surface water is not its natural habitat, the germination of spores is not expected and B*acillus thuringiensis* subsp*. kurstaki* is unlikely to have the potential to multiply (and produce crystal proteins) in freshwater environments even under higher nutrient/oxygen conditions. A new adapted OECD 309 study which investigated the survivability of spores in surface water is available and summarised below (Zetzmann, 2023). The study investigated the survivability of viable *Btk* strain ABTS-351 spores present in the DiPel Biological Insecticide Technical Powder in aerobic natural surface water collected from a pond in Germany. Over a 10 day incubation period, the overall mean number of viable spores of *Btk* strain ABTS-351 decreased by >50% when held in the dark at 20oC and the study reported at 10 days after treatment, a 2.21 fold decrease in survivability. The results confirm that germination and proliferation of *Btk* spores is unlikely in natural surface water. A summary of the study is presented below.

|  |  |
| --- | --- |
| Data point addressed | IIIM 9.2.1/01 |
| Author(s) (year) | Zetzmann, M. (2023) |
| Title | DiPel Biological Insecticide Technical Powder: Survivability in Surface Water |
| Report number | S22-04102 |
| EFSA Study Identification | EFSA-2022-00011996 |
| Test facility | Eurofins Aquatic Ecotoxicology GmbH, Germany |
| Published | No |
| Test guideline | OECD 309 adapted |
| Deviations | None. However, this is an adaptation of the guidance to determine the survivability of the test item in aerobic natural surface water over 10 days. |
| GLP | Yes, conducted under GLP/Officially recognised testing facilities |

**Executive Summary**

The survivability of DiPel Biological Insecticide Technical Powder in aerobic natural surface water in the dark and at 20oC, was determined by measuring the content of viable spores of *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 after 0, 2, 4, 7 and 10 days of incubation in three replicate samples. Untreated control samples were incubated alongside treated samples.

Samples were clear and transparent and no turbidity was observed. The number of viable spores reduced from 1.14E+06 CFU/mL at 0 days after treatment (0 DAT) to 5.17E+05 CFU/mL at 10 DAT equating to a 55% (or 2.2 fold) reduction. The study concluded that the survivability of the test item plateaued at between 4 and 10 days; with a % survivability of approximately 40 to 45%.

1. **MATERIALS AND METHODS**

**A. Materials**

**Test item**: DiPel Biological Insecticide Technical Powder

Batch no.: 332501V930

Active ingredient: *Bacillus thuringiensis* subsp*. kurstaki* strain ABTS-351

Purity analysed: 91% Unit/mg

Appearance / colour: Solid/brown

Stock solutions of the test item were prepared in phosphate buffer (Butterfield’s buffer) and the suspension homogenised for further dilution.

**Test system**

Natural aerobic surface water was collected from a pond located within a nature reserve in Rhineland-Palatinate, Germany. The sampling site and water characteristics are detailed in Table 9.2.1-1. The water was sampled from the surface at a depth of 5 to 10 cm. The sampling location is in an area not influenced by effluents or human activity and has no known pesticide history. Before use the water was filtered through a 100 µm sieve to remove coarse particles. The water was stored at approximately 4°C with aeration and the experiment started 3 days after collection.

**Table 9.2.1-1 Physical and chemical characteristics of the Natural Water**

|  |  |
| --- | --- |
| Characterisation | Natural Water |
| Type of water | Pond |
| Source | Rhineland-Palatinate, 67374 Hanhofen, Germany, (49°18'42.84"N, 8°19'10.5744"E) |
| Date of Sampling | 22 July 2022 |
| Depth of sampling | Top 5-10 cm |
| Temperature (˚C)A | 24.6 |
| pH A | 8.09 |
| Redox potential (mV) A | 223 |
| Oxygen content below water surface (mg/L) A | 8.03 |
| Oxygen content (water/sediment interface) (mg/L) A | 7.66 |
| Odour | Neutral |
| Colour | Colourless/ no turbidity |
| Total organic carbon B (mg/L) | 8.6 |
| Dissolved organic carbon B (mg/L) | 8.2 |
| Biological oxygen demand B (mg/L O2) | 7.6 |
| Total Nitrogen (mg/L) | 2.1 |
| Total Phosphorus (mg/L) | 0.4 |
| Dissolved orthophosphate (mg/L) B | 0.4 |
| Total Nitrite (mg/L) B | <0.1 |
| Total Nitrate (mg/L) B | <0.1 |
| Nitrogen (ammoniacal) distillation (mg/L) B | 0.2 |
| Hardness (mg equivalent CaCO3/L) | 210 |

A Measured at sampling site; B measured in laboratory.

**B. STUDY DESIGN AND METHODS**

**1. Experimental Conditions**

The test item, DiPel Biological Insecticide Technical Powder was added to 100 mL aerobic natural surface water (test medium) in glass flasks at a nominal test concentration of 1 x 106 CFU/mL and incubated for 10 days in the dark at 20 ± 2°C with continuous agitation. Test flasks were closed with a PU plug to allow gas exchange. Control flasks only containing the surface water test medium were incubated as blank controls.

The content of viable spores of the active ingredient *Bacillus thuringiensis* subsp. *kurst*aki strain ABTS-351 was determined in three replicate test systems and one control after 0, 2, 4, 7 and 10 days to assess survivability. Samples were taken from each flask and an aliquot taken and diluted with phosphate buffer (Butterfield’s Buffer) and the dilutions incubated in a water bath at 80 ± 1°C for 10 minutes to remove vegetative cells (leaving only the bacterial spores). Samples were cooled to room temperature and further diluted with buffer solution and homogenised. For each replicate five tryptone soya agar plates were inoculated with the diluted test solution and incubated at 30-35°C for 18 hours in order to calculate a mean spore load for each replicate flask. Incubation colonies were counted and colony counts below 10 or over 150 were excluded from the evaluation. Colonies non-typical and with a morphology different to *B. thuringiensis* subsp. *kurstaki* strain ABTS-351 were excluded from the evaluation.

**II. RESULTS AND DISCUSSION**

Samples taken at each time point resulted in overall mean temperatures of 20.9 to 21.3°C with mean pH values of 8.37 to 8.39. The oxygen content of test solution declined from 8.15 to 8.35 mg/L at 0 days after treatment (DAT) to 5.55 to 5.88 mg/L at 2 DAT and then slightly increased measuring 5.91 to 6.03 mg/L at the end of incubation (10 DAT). No turbidity in samples was observed during the test period.

Table 9.2.1-2 details the overall viable mean spore load at each time point. A 2.21-2.52 fold reduction was observed between 4 and 10 days when compared to the counts at 0 days after treatment (0 DAT).

Note that in the study report, the % survivability was calculated based on Log10 [CFU/mL] values and so have been re-calculated based on the direct number of spores.

**Table 9.2.1-2** **Summary of results for Survivability of *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 in natural surface water following treatment with DiPel Biological Insecticide Technical Powder**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Timing**  **[d]** | **Concentration *Bacillus thuringiensis subsp. kurstaki* strain ABTS-351 found [CFU/mL] 1)** | | **% Reduction 3)** | **% Survivability3)** | **X fold reduction** |
| **Control group** | **Treated group** | **Treated group** | **Treated group** | **Treated group** |
| Before application | 0.00E+00 | 0.00E+00 | -- | -- | -- |
| 0 | 8.00E+002) | 1.14E+06 | -- | -- | -- |
| 2 | 0.00E+00 | 9.31E+05 | 18.3 | 81.7 | 1.23 |
| 4 | 0.00E+00 | 4.75E+05 | 58.3 | 41.7 | 2.41 |
| 7 | 4.00E+002) | 4.53E+05 | 60.3 | 39.7 | 2.52 |
| 10 | 0.00E+00 | 5.17E+05 | 54.6 | 45.4 | 2.21 |

1) Mean value of three replicates

2) Singular colonies were observed on some plates. This is still less than the maximum acceptable count of average 2 colonies per 5 plates.

3) In the study report, the % survivability of spores was calculated based on Log10 [CFU/mL] values. The results presented above show the % reduction and subsequent % survivability calculated based on the concentration of viable spores presented in Column 3 of the table (treated group) and are relative to the concentration at 0 days after application.

**III. CONCLUSION**

The survivability of the test item in natural surface water appeared to plateau at ~ 40 - 45% after 4 days.

(Zetzmann, M., 2023)

|  |
| --- |
| **Assessment and conclusion by applicant:**  The study is acceptable. It confirms that viable *Btk* strain ABTS-351 spores do not increase during incubation in natural surface water and the results indicate a general decline in spore viability over a period of 10 days. |

|  |
| --- |
| **Assessment and conclusion by RMS:**  The submitted study was accepted.  No deviation was noted.  The initial measured concentration of viable spores of *Bacillus thuringiensis subsp. kurstaki* strain ABTS-351 was 1.14E+06 CFU/mL. After 10 days, the concentration was 5.17E+05 CFU/mL.  Samples were clear and transparent, no turbidity was observed. |

In addition, the RAR (2020) and RMS comments also detail that there are numerous literature references available on *Bt* including *Btk*, which all confirm the absence of germination and growth outside the target insect and restricted persistence in all relevant environmental compartments including water. In water, there will be a high variability as to if bacterial cells and spores of *Btk* survive and this will be influenced by many factors (biological, chemical and physical). Bacterial populations will be subject to natural competition and predation by bacteriophage, protozoans and other lower animal forms (RAR, 2020). As *Btk* is not a natural inhabitant of aquatic environments it is unlikely to find optimal conditions which are nutrient rich (e.g. water are poor in organic carbon). Several open literature publications where aquatic microbial community shifts in response to organic matter influx have been studied and members of the Phylum Firmicutes, which *B. thuringiensis* species belong, have not been observed to be among the dominant microbial populations which responded to organic matter influx. Response to organic matter availability have rather been consistently dominated by microbial populations within the Phylum *Proteobacteria* (including Class *Alphaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria*), *Bacteriodetes*, *Verrucomicrobia*, *Actinobacteria*, and *Chloroflexi*. (Woodhouse *et al*., 2016; Bergauer *et al*., 2017; Bizic-Ionescu *et al*., 2018; Zhou *et al*., 2018).

Zhou *et al*. (2018) stressed the importance of considering biotic interactions such as competition and predation when investigating the link between microbial ecological behaviour and the phases of organic matter degradation in the environment. Bergauer *et al*. (2017) found that the heterotrophic prokaryotic community tend to utilise similar organic compounds further suggesting that microbial communities would need to compete for nutrients in aquatic environments. Therefore, in the unlikely event that *Btk* proliferates in freshwater environments under higher nutrient conditions, it would have to compete for nutrients with several tens of thousands other microbial species within the dominant phyla, most of which are better adapted to aquatic settings, including those within its own phylum. For perspective, according to Volume 3 of the Bergey’s Manual® of Systematic Bacteriology, *Bacillus thuringiensis* is only one of 1346 known species within the phylum Firmicutes, with itself comprising of several subspecies and strains. Therefore, any *Bacillus thuringiensis* subsp*. kurstaki* strain ABTS-351 reaching the aquatic environment e.g., through spray drift during application, will encounter unfavourable conditions, which will lead to a fast decline of the population. This was concluded by the RMS who commented that proliferation of this bacterial species in natural water bodies is not expected to occur, and population size will decline upon hostile environmental conditions; long term survival in water is very unlikely (RAR, 2020).

Brief summaries of studies referred to above are provided below:

|  |  |
| --- | --- |
| Data point addressed | IIIM 9.2.1/02 |
| Authors(s) year | Woodhouse, J.N., Kinsela, A.S., Collins, R.N., Bowling, L.C., Honeyman, G.L., Holliday, J.K., and Neilan, B.A. (2016) |
| Title: | Microbial communities reflect temporal changes in cyanobacterial composition in a shallow ephemeral freshwater lake |
| Report No. | The ISME Journal (2016) 10, p. 1337-1351 |
| Test facility | Not applicable |
| Published | Yes |
| Test guidelines: | Not applicable |
| Deviations | Not applicable |
| GLP: | No |

Aim

To address how abiotic and biotic factors combine and interact during cyanobacterial blooms in freshwater systems.

Methods

The authors performed routine monitoring over a 6-month period and performed in situ measurements, elemental analyses, identification analysis and enumeration of microbial populations and species using 16S rRNA amplicon sequencing at five sites across Yanga Lake, Australia.

Findings

The study highlights the dynamic nature of freshwater microbial communities, during periods of elevated cyanobacterial blooms. The authors observed the influence of several abiotic and biotic variables on microbial populations. Species-specific associations between cyanobacteria and bacterioplankton, including the free-living *Actinobacteria*, *Bacteroidetes*, *Betaproteobacteria* and *Verrucomicrobia,* were also identified.

Conclusion

It was concluded that changes in the abundance and nature of freshwater cyanobacteria are associated with changes in the diversity and composition of lake bacterioplankton.

|  |
| --- |
| **Assessment and conclusion by RMS:**  The submitted report was accepted as an additional/supportive information. |

|  |  |
| --- | --- |
| Data point addressed | IIIM 9.2.1/03 |
| Authors(s) year | Bergauer, K., Fernandez-Guerra, A., Garcia, J.A.L., Sprenger, R.R., Stepanauskas, R., Pachiadaki, M.G., Jensen, O.N., and Herndl, G. J. (2017) |
| Title: | Organic matter processing by microbial communities throughout the Atlantic water column as revealed by metaproteomics |
| Report No. | Proc Natl Acad Sci U S A. (2017) 115(3), p. 445-447 |
| Test facility | Not applicable |
| Published | Yes |
| Test guidelines: | Not applicable |
| Deviations | Not applicable |
| GLP: | No |

Aim

The authors sought to answer two questions:(1) Are changes in the phylogenetic composition of the microbial community tightly or loosely linked to changes in the type and abundance of transporter proteins for organic matter (OM) throughout the water column? (2): Does the distribution of transporter protein indicate major changes in the OM compound classes used as substrate by the microbial communities between surface and bathypelagic waters?

Methods

The authors integrated metaproteomic, metagenomic, and single-cell genomic analyses to elucidate protein expression patterns of microbial communities from the euphotic zone (100 m) to the dark ocean (300–4,050 m) along a large latitudinal range (67°N to 49°S) in the Atlantic Ocean in response to organic nutrient processing. They focused on the spatial distribution and abundance of expressed transporters as key mechanisms for the uptake of essential nutrients, including the primary active ATP-binding cassette (ABC) transporters, as well as the secondary active tripartite ATP-independent periplasmic transporters (TRAP-Ts), the tripartite tricarboxylate transporters (TTTs), and the TonB-dependent transporters (TBDTs).

Findings

By identifying which compounds of the organic matter pool are absorbed, transported, and incorporated into microbial cells, intriguing insights into organic matter transformation in the deep ocean emerged. On average, solute transporters accounted for 23% of identified protein sequences in the lower euphotic and ∼39% in the bathypelagic layer, indicating the central role of heterotrophy in the dark ocean. In the bathypelagic layer, substrate affinities of expressed transporters suggest that, in addition to amino acids, peptides and carbohydrates, carboxylic acids and compatible solutes may be essential substrates for the microbial community. Key players with highest expression of solute transporters were *Alphaproteobacteria*, *Gammaproteobacteria*, and *Deltaproteobacteria*, accounting for 40%, 11%, and 10%, respectively, of relative protein abundances.

Conclusion

The in-situ expression of solute transporters indicates that the heterotrophic prokaryotic community is geared toward the utilization of similar organic compounds throughout the water column, with yet higher abundances of transporters targeting aromatic compounds in the bathypelagic realm.

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| **Assessment and conclusion by RMS:**  The submitted report was accepted as an additional/supportive information. |

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| Data point addressed | IIIM 9.2.1/04 |
| Authors(s) year | Zhou, J.; Richlen, M.L., Sehein, T.R., Kulis, D.M., Anderson, D.M., and Cai, Z. (2018) |
| Title: | Microbial Community Structure and Associations During a Marine Dinoflagellate Bloom |
| Report No. | Front. Microbiol. (2018), 9(1201), p. 1-21 |
| Test facility | Not applicable |
| Published | Yes |
| Test guidelines: | Not applicable |
| Deviations | Not applicable |
| GLP: | No |

Aim

To explore the population dynamics of microorganisms during an algal bloom, and to identify and characterize correlations among microbial taxa using network analysis and ultimately further understanding of algal-bacteria interactions.

Method

The authors hypothesized that microbial community structure and succession is linked to specific bloom stages, thus driving complex interactions among taxa comprising the phycosphere environment. High throughput sequencing and association networking methods was used to analyse prokaryotic and eukaryotic microbial community dynamics during a natural dinoflagellate bloom to better characterize patterns of interaction and related ecological behaviour during harmful algal bloom (HAB) events.

Findings

Within the bacterial community, *Gammaproteobacteria* and *Bacteroidetes* were predominant during the initial bloom stage, while *Alphaproteobacteria*, *Cyanobacteria,* and *Actinobacteria* were the most abundant taxa present during bloom onset and termination. In the archaea biosphere, methanogenic members were present during the early bloom period while the majority of species identified in the late bloom stage were ammonia-oxidizing archaea and *Halobacteriales*. Dinoflagellates were the major eukaryotic group present during most stages of the bloom, whereas a mixed assemblage comprising diatoms, green algae, rotifera, and other microzooplankton were present during bloom termination. Temperature and salinity were key environmental factors associated with changes in bacterial and archaeal community structure, respectively, whereas inorganic nitrogen and inorganic phosphate were associated with eukaryotic variation.

Network analysis showed that *Maxillopoda*, *Spirotrichea*, Dinoflagellata, and *Halobacteria* were keystone taxa within the positive correlation network, while *Halobacteria*, *Dictyochophyceae*, *Mamiellophyceae*, and *Gammaproteobacteria* were the main contributors to the negative-correlation network. The positive and negative relationships were the primary drivers of mutualist and competitive interactions that impacted algal bloom fate, respectively. Functional predictions showed that blooms enhance microbial carbohydrate and energy metabolism.

Conclusion

Microbial community structure is strongly linked to bloom progression. However, specific drivers of community interactions and responses are still poorly understood.

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| **Assessment and conclusion by RMS:**  The submitted report was accepted as an additional/supportive information. |

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| Data point addressed | IIIM 9.2.1/05 |
| Authors(s) year | Bizic-Ionescu, B., Ionescu, D., and Grossart, H. (2018) |
| Title: | Organic Particles: Heterogeneous Hubs for Microbial Interactions in Aquatic Ecosystems |
| Report No. | Front. Microbiol. (2018), 9(2569), p. 1-15 |
| Test facility | Not applicable |
| Published | Yes |
| Test guidelines: | Not applicable |
| Deviations | Not applicable |
| GLP: | No |

Aim

To investigate microbial colonisation of organic particles in the German freshwater lake Stechlin.

Method

A transcriptomic approach was used to assess the role of stochastic events on initial microbial colonisation of particles. Replicate samples consisting of 3-4 particles of identical source and age were used. Using flow through rolling tanks, long-term experiments were conducted at near *in situ* conditions minimizing the biasing effects of closed incubation approaches often referred to as “the bottle-effect.” Furthermore, the authors asked whether gene expression from microbial communities colonising the particles corroborates rapid changes in carbon-quality.

Findings

Active microbial communities were highly heterogeneous despite an identical particle source, suggesting random initial colonisation of organic particles. The study also suggested that in nature, changes in particle-associated community related to carbon availability are slower (days to weeks) due to constant supply of labile, easy degradable organic matter. Initially, random particle colonisation seems to be subsequently altered by multiple organismic interactions (*e.g.* competition and antagonism) shaping microbial community interactions and functional dynamics.

The dominant active microbial groups consisted of members of the Family *Flavobacteriaceae, Pseudomonadaceae, Cytophagaceae, Enterobacteraceae, Moraxellaceae, Sphingomonadaceae*, and *Bacteroidaceae*. The active eukaryotic community from the long-term incubations of single particles and all pooled lake particles was dominated by *Plectosphaerallaceae*, a family of fungi. Viruses made up only a small portion of the reads from the short-term incubated single particles and consisted of *Myoviridae*, *Siphoviridae*, and unclassified viruses.

Conclusion

Single particles represent complex micro-niches in the water column which are randomly colonised by a diverse microbial assemblage from the surrounding water. Competitive and antagonistic activities on particles, between all prokaryotic and eukaryotic entities play an essential, yet underestimated role in determining dynamics of particle-associated community composition.

Crystal Proteins

The endotoxins do not persist or accumulate in water.

For the crystal proteins, it was agreed that the endpoints for the surface water exposure assessment should be read across degradation and adsorption endpoints between the different crystal proteins from the available dataset that contains measured endpoints from only a subset of the different ẟ-endotoxins or crystal protein test material. For the DT50 in water a value of 28 days was agreed upon (the longest DT50 from non-sterile water). EFSA considered that this information was also likely to cover the Vip and Sip proteins if produced.

DT50 properties of the Cry-protein are summarised in the table below:

|  |  |
| --- | --- |
| **Water/Sediment** | |
| **DT50 (days)** | **Experiment/Cry Protein** |
| 0.9 | *Bti* protoxin, laboratory microcosms |
| 1.5 | *Bti* protoxin, laboratory microcosms |
| 7 | *Bti* protoxin river |
| **28\*** | *Bti* protoxin river |
| 130.8 | Cry1Ac artificial ‘natural’ water, sterile hydrolysis |
| 93.7 | Cry1Ac artificial ‘natural’ water, sterile hydrolysis |

\* used as end point in environmental exposure assessments

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| **Assessment and conclusion by RMS:**  The submitted report was accepted as an additional/supportive information. |

### IIIM 9.2.2 Predicted Environmental Concentrations in water (PECSW) for the Formulation, Active Substance and Crystal Proteins

For the environmental exposure assessment for the formulation and active substance only the spray drift pathway is relevant and therefore, during the EU evaluation of *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 the Rautmann *et al.* (2001)[[3]](#footnote-4) drift values were used for the surface water exposure assessment and as such, have been used for calculations for the requested product authorisation. Therefore, the approach presented herewith has followed the approach detailed in the EFSA Journal 2021; 19(10):6879. Predicted Environmental Concentrations (PECSW) and Predicted Environmental Density (PEDSW) values in surface water have been calculated for the formulated product, the microorganism *Btk* strain ABTS-351 and the crystal proteins. Calculations are presented for both single and multiple applications considering the highest individual application rate and the maximum number of four applications to cover the risk envelope for all uses. For multiple applications, the total seasonal dose was used as a single application (i.e., assuming no reduction in the microbe populations or degradation between applications) and the drift value relevant to the number of applications per season used. For the formulated product, PECSW values were calculated for only a single application dose as it is considered that upon application, the formulation will break down to its constituent parts.

The GAP specifies aerial and ground spray applications. For aerial applications, the spray drift values used for FOCUS Step 1 and 2 were used (which are the values from the AgDrift model (SDTF, 1999) corrected to a distance of 3 m from the edge of the treated field; FOCUS, 2015[[4]](#footnote-5)). For ground application to forests, trees and shrubs the conservative spray drift values for pome/stone fruit (application early; spray drift value of 29.2% (single applications) were used as a worst-case surrogate crop for all uses. Using pome/stone fruit early application drift values (instead of late applications which takes account of the canopy) is conservative as the GAP specifies coniferous trees (for which the canopy will be present throughout the year) and for deciduous trees, it is considered that most applications will be made post-flowering when the most canopy is present. The calculations are considered to cover the risk envelope for applications to deciduous and coniferous forest, trees and shrubs with a large margin of safety.

The PEC/PEDSW values presented in the EFSA Journal 2021; 19(10):6879 consider both a ditch volume of 210 L/m2 and 300 L/m2, respectively. A ditch of 210 L/m2 is based upon the Netherlands National Surface Water Model TOXSWA v1.2 where the ditch has sloping sides (Beltman and Adriaanse 1999[[5]](#footnote-6)), whereas a volume of 300 L/m2 is based upon the FOCUS dimensions for a rectangular 1 m section of ditch which is 1 m wide and 0.3 m deep. The FOCUS dimensions of the ditch are detailed in the FOCUS surface water guidance and are used for FOCUS Step 1-2 calculations, which consider a volume of 300 L/m2. Following the methodology presented in the EFSA Journal, the two sets of calculations have been performed; however, as the PEC/PEDSW values calculated for a ditch with a volume of 210 L/m2 are for the Netherlands only (and therefore, presented for information only) they are detailed in Appendix 3. For EU MS (with the exception of the NL), it is the calculations based on a volume of 300 L/m2 that are relevant.

The maximum GAP is for four applications of Foray® 76B at 2.5 L formulated product/ha with a minimum interval of 5 days which will cover the risk envelope for all GAPs for calculations for the active substance and the crystalline proteins. PEC values have also been calculated for the formulated product based on a single application as it is considered that upon application, the formulated product will breakdown to its constituent parts. An application of 3 L formulated product/ha is the highest individual dose specified in the GAP for applications to deciduous forest (2 x 619 g a.s./ha; 14-day interval) and therefore, for single applications, this maximum individual rate of application has been used.

Calculations for the predicted environmental density consider a maximum individual application rate of the microorganism in the formulated product of 5.07 × 1013 CFU/ha (equivalent to 3.0 L product/ha) and multiple treatments of four applications of 4.23 × 1013 CFU/ha (equivalent to 2.5 L product/ha). These application rates are based on a maximum content of 1.69 × 1013 CFU/L of the microorganism in the formulated product Foray® 76B.

For the crystal proteins associated with the microorganism, calculations for spray drift have also been performed using the Rautmann (or FOCUS Step 1-2) drift values considering between applications either no degradation (total seasonal dose used) or degradation (water/sediment DT50 value of 28 days) and a worst-case GAP of 4 applications with a minimum 5-day interval to cover the risk envelope for all uses(Beltman and Adriaanse, 1999). The crystalline proteins are present both inside and outside viable spores. As some crystal proteins may be present outside the spores within the formulated product, following the methodology performed during the EU evaluation of the MPCA, FOCUS Steps 1-2 calculations have been performed. The calculations for the crystal proteins consider that 12.7% (w/w) of the MPCA within the formulated product is present as crystal protein. Calculations for the crystal proteins were performed using the DT50 (soil) of 41.3 days, Koc of 1000 mL/g and DT50 (water/sediment) of 28 days as agreed during the expert meeting in March 2020.

Applications will be made to forests, trees and shrubs and therefore, there will be significant foliar interception. During the EU evaluation, no crop interception was considered and therefore, calculations presented here have also assumed no crop interception. Consequently, similar to the soil exposure assessment, the resulting FOCUS Step 2 PEC values for the crystal proteins in the aquatic environment represent a very worst-case.

*Calculation of PEDSW and PECSW*

The loading to surface water via the spray drift pathway was calculated using the spray drift tables of Rautmann *et al.* (2001) or FOCUS 1-2 (aerial applications) and the following formulae:

Where:

PECSW (ini) PEC in surface water [µg/L] immediately following a single application or following multiple applications with no degradation taken into account

PEDSW (ini) PED in surface water [CFU/L] immediately following a single application or following multiple applications with no degradation taken into account

A Application rate [in CFU/m2 or µg/m2]

Dep rate % spray drift

V*sw* Water volume of water body per 1 m length of field

Agronomic input parameters (all calculations) are presented in Table 9.2.2-1.

**Table 9.2.2-1 Agronomic input parameters related to application of *Btk* for PECSW/(SED) calculations**

| **Use No.** | **All Uses** |
| --- | --- |
| Crop | Pine trees, deciduous/coniferous forest, shrubs, ornamental trees and plants. |
| FOCUS Crop | Aerial Spray / Pome fruit (early application) |
| Application rate  Formulated product [g/ha]\*\*\* | 4 × 2800 g Foray® 76B/ha  (1 × 3360 g Foray® 76B/ha\*\*\*\*) |
| Application rate  Active substance [g/ha] | 4 × 520 g MPCA/ha |
| Application rate  Active substance [CFU/ha] | 4 × 4.23×1013 CFU/ha |
| Application rate  Crystal Proteins [g/ha] | 4 × 66.04 g CryP/ha |
| Number of applications/interval | 4/5 |
| Total Annual Application dose Active substance [g/ha] | 1 × 2080 g MPCA/ha |
| Total Annual Application rate  Crystal Proteins [g/ha] | 1 × 264.16 g CryP/ha |
| Total Annual Application dose *Btk* [CFU/ha] | 1 × 1.69×1014 CFU/ha |
| Spray drift values for single/multiple applications [%] | \*Aerial: 33.2 / 33.2 (FOCUS Step 1-2)  \*\*Ground Spray: 29.2 / 23.61 (based on pome fruit early application) |
| Default distance to water body for spray drift calculations [m] | 3 |
| Application window at Step 1-2 | South and North EU;  Oct-Feb  Mar-May Jun-Sep |
| Crop interception [%] | 0% |
| Models used for calculation | FOCUS and Rautmann Drift values, Steps 1-2 in FOCUS v 3.2 |

\* Spray drift value from FOCUS STEP 1-2 software;

\*\* Rautmann Spray drift value for pome early applications

\*\*\* Application rate of the product considers a density of Foray® 76B of 1.12 g/cm3

\*\*\*\*The maximum single application is 3.0 L product/ha whereas the maximum seasonal dose arises from four applications of 2.5 L product/ha.

**FOCUS Step 1-2 Calculations**

The “Steps 1-2 in FOCUS” calculator, version 3.2, was used for the Step 1 and 2 calculations. Substance specific input parameters were taken from the List of Endpoints (EFSA, 2021) or the RAR (2020) and are detailed in Table 9.2.2-2.

**Table 9.2.2-2 Summary of input parameters for the Cry Proteins associated with *Btk* for PECSW and PECSED FOCUS Step 1-2 calculations**

|  |  |  |
| --- | --- | --- |
| **Parameter** | **Parameter value** | **Remarks** |
| Entry routes into surface water | Spray drift, Drainage, Run-off | - |
| **Physico-chemical parameters** | | |
| Water solubility [mg/L] | 1000 | At 20°C, worst-case assumption |
| **Degradation in soil** | | |
| DT50 (soil) [d] | 41.3 | Agreed Endpoint, Maximum aerobic soil DT50 of 41.3 d at 20°C (EFSA. 2021) |
| **Sorption to soil** | | |
| KOC [L/kg] | 1000 | Agreed Endpoint, Representative worst-case (EFSA, 2021) |
| **Degradation in water-body** | | |
| DT50 total aquatic system [d] (Step 1 & 2) | 28 | Longest value at 20°C (EFSA, 2021) |
| DT50 water [d] (Step 2) | 28 | Longest value at 20°C (EFSA, 2021) |
| DT50 sediment [d], (Step 2) | 28 | Longest value at 20°C (EFSA, 2021) |

Predicted Environmental Densities in surface water (PEDSW) and Predicted Environmental Concentrations in surface water (PECSW) via the drift pathway and calculated using the Rautmann *et al.* (2001) drift values or the FOCUS Step 1-2 drift values for aerial applications are detailed in Table 9.2.2-3.

**Table 9.2.2-3** **PEDSW and PECSW values for the spray drift pathway (using the FOCUS / Rautmann Drift values)**

| **Use** | **Drift Crop** | **Rate of Application** | **Drift values [%]** | **Ditch volumetry** | **PECSW (single application)** | **PECSW (multiple applications)** | **Unit** |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Formulated product (PECSW)** | | | | | | |
| All Uses | Aerial | 1 × 3360 g Foray® 76B  / ha | 33.2 | 300 L/m2 | 371.84 \*\*\* | - | µg/L |
| Fruit trees, early application\*\* | 1 × 3360 g Foray® 76B  / ha | 29.2 | 300 L/m2 | 327.04 \*\*\* | - | µg/L |
|  | **Active substance (PECSW)** | | | | | | |
| All Uses | Aerial | 4 × 520 g MPCA/ha  (1 × 619 g MPCA/ha) | 33.2 / 33.2 | 300 L/m2 | 68.50 \*\*\* | 230.19 | µg/L |
| Fruit trees, early application\*\* | 4 × 520 g MPCA/ha  (1 × 619 g MPCA/ha) | 29.20 / 23.61 | 300 L/m2 | 60.25 \*\*\* | 163.70 | µg/L |
|  | **Active Substance (in Colony Forming Units; CFU; PEDSW)** | | | | | | |
| All Uses | Aerial | 4 × 4.23 × 1013 CFU/ha  (1 × 5.07 × 1013 CFU/ha) | 33.2 / 33.2 | 300 L/m2 | 5.61 × 106 \*\*\* | 1.87 × 107 | CFU/L |
| Fruit trees, early application\*\* | 4 × 4.23 × 1013 CFU/ha  (1 × 5.07 × 1013 CFU/ha) | 29.20 / 23.61 | 300 L/m2 | 4.93 × 106 \*\*\* | 1.33 × 107 | CFU/L |
|  | **Cry proteins (PECSW)\*\*** | | | | | | |
| All Uses | Aerial | 4 × 66.04 g CryP/ha  (1 × 78.61 g CryP/ha) | 33.2 / 33.2 | 300 L/m2 | 8.70 \*\*\* | 24.51\* (29.23) | µg/L |
| Fruit trees, early application\*\* | 4 × 66.04 g CryP/ha  (1 × 78.61 g CryP/ha) | 29.20 / 23.61 | 300 L/m2 | 7.65 \*\*\* | 17.43\* (20.79) | µg/L |

\* PECsw (spray drift pathway) considering degradation between the 4 applications (DT50 = 28 days). In brackets, PECSW does not take into account the degradation between applications (as worst-case). Total seasonal dose was used;

\*\* Drift values for fruit trees or pome/stone fruit (early application) used as a surrogate for application to forests and ornamental trees which will also cover the risk envelope for shrubs;

\*\*\* A maximum individual dose of 3.0 L product/ha or 619 g MPCA/ha was used for single application calculations.

Predicted Environmental Concentrations in surface water (PECSW) and sediment (PECSED) for the crystal proteins calculated using FOCUS Steps 1-2 are presented in Table 9.2.2-4 and Table 9.2.2-5, respectively.

**Table 9.2.2-4** **FOCUS Step 1 and 2 PECSW values for the Crystal Proteins**

| **Use** | **FOCUS Crop** | **Rate of Application** | **FOCUS Scenario** | **Ditch volumetry** | **PECSW (single appl.)** | **PECSW (multiple appl.)** | **Unit** |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Cry proteins; Step 1** | | | | | | |
| All Uses | Aerial | 4 × 66.04 g CryP/ha  (1 × 78.61 g CryP/ha\*\*) | Step 1 | 300 L/m2 | 19.93 | 66.97 | µg/L |
| Pome/stone fruit  (early application) | Step 1 | 300 L/m2 | 18.88 | 63.45 | µg/L |
|  | **Cry proteins; Step 2** | | | | | | |
| All Uses | Aerial | 4 × 66.04 g CryP/ha  (1 × 78.61 g CryP/ha\*\*) | NEU: O-F | 300 L/m2 | 9.43 | 27.38 | µg/L |
| NEU: M-M | 300 L/m2 | 8.70 | 18.01 | µg/L |
| NEU: J-S | 300 L/m2 | 8.70 | 18.01 | µg/L |
| SEU: O-F | 300 L/m2 | 8.70 | 24.26 | µg/L |
| SEU: M-M | 300 L/m2 | 8.70 | 24.26 | µg/L |
| SEU: J-S | 300 L/m2 | 8.70 | 21.13 | µg/L |
| All Uses | Pome/stone fruit  (early application) | 4 × 66.04 g CryP/ha  (1 × 78.61 g CryP/ha\*\*) | NEU: O-F | 300 L/m2 | 8.92 | 23.98 | µg/L |
| NEU: M-M | 300 L/m2 | 7.65 | 14.61 | µg/L |
| NEU: J-S | 300 L/m2 | 7.65 | 14.61 | µg/L |
| SEU: O-F | 300 L/m2 | 7.87 | 20.86 | µg/L |
| SEU: M-M | 300 L/m2 | 7.87 | 20.86 | µg/L |
| SEU: J-S | 300 L/m2 | 7.65 | 17.73 | µg/L |

\* The FOCUS crop pome/stone fruit (early application) used as a surrogate for application to forests, trees which will also cover the risk envelope for shrubs;

\*\* A maximum individual dose of 3.0 L product/ha or 619 g MPCA/ha was used for single application calculations.

**Table 9.2.2-5** **FOCUS Step 1 and 2 PECSED values for the Crystal Proteins**

| **Use** | **Crop** | **Rate of Application** | **FOCUS Scenario** | **Ditch volumetry** | **PECSED (single appl.)** | **PECSED**  **(multiple appl.)** | **Unit** |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Cry proteins; Step 1** | | | | | | |
| All Uses | Aerial | 4 × 66.04 g CryP/ha  (1 × 78.61 g CryP/ha\*\*) | Step 1 | 300 L/m2 | 145.93 | 490.37 | µg/kg |
| Pome/stone fruit  (early application) | Step 1 | 300 L/m2 | 141.54 | 475.63 | µg/kg |
|  | **Cry proteins; Step 2** | | | | | | |
| All Uses | Aerial | 4 × 66.04 g CryP/ha  (1 × 78.61 g CryP/ha\*\*) | NEU: O-F | 300 L/m2 | 84.16 | 245.26 | µg/kg |
| NEU: M-M | 300 L/m2 | 53.43 | 153.80 | µg/kg |
| NEU: J-S | 300 L/m2 | 53.43 | 153.80 | µg/kg |
| SEU: O-F | 300 L/m2 | 73.92 | 214.77 | µg/kg |
| SEU: M-M | 300 L/m2 | 73.92 | 241.77 | µg/kg |
| SEU: J-S | 300 L/m2 | 63.68 | 184.28 | µg/kg |
| All Uses | Pome/stone fruits  (early application) | 4 × 66.04 g CryP/ha  (1 × 78.61 g CryP/ha\*\*) | NEU: O-F | 300 L/m2 | 80.19 | 218.42 | µg/kg |
| NEU: M-M | 300 L/m2 | 49.46 | 126.97 | µg/kg |
| NEU: J-S | 300 L/m2 | 49.46 | 126.97 | µg/kg |
| SEU: O-F | 300 L/m2 | 69.95 | 187.94 | µg/kg |
| SEU: M-M | 300 L/m2 | 69.95 | 187.94 | µg/kg |
| SEU: J-S | 300 L/m2 | 59.70 | 157.45 | µg/kg |

\* The FOCUS crop pome/stone fruit (early application) used as a surrogate for application to forests, trees which will also cover the risk envelope for shrubs;

\*\* A maximum individual dose of 3.0 L product/ha or 619 g MPCA/ha was used for single application calculations.

|  |  |
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| Conclusion (PECsw and PECsed) (active substance/relevant metabolite): IIIM 9.7.1/01 | The submitted approach and justification was accepted.  The used endpoints were accepted as representing the worst case.  Use of FOCUS / Rautmann Drift values for spray drift assessment was accepted. |

## IIIM 9.3 Fate and behaviour in groundwater

### IIIM 9.3.1 Predicted Environmental Concentrations in Groundwater (PECGW)

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| --- | --- |
| zRMS  Comments: | The submitted PECgw assessment report and justification were accepted.  The used endpoints in PECgw assessment were accepted as representing the worst case.  In modelling the models FOCUS PEARL 5.5.5 and FOCUS PELMO 6.6.4 were used.  The risk envelope approach and surrogate crop were accepted. |

Mobility of spores of *B. thuringiensis* subsp. *kurstaki* including *Btk* ABTS-351 can be considered limited (EFSA, 2021). *Bacillus thuringiensis* is not expected to be mobile in soil and is unlikely to leach through soils to groundwater. The potential for groundwater contamination following application of the Foray® 76B formulation is negligible. During the EU evaluation of *Bacillus thuringiensis* subsp. *kurstaki* including *Btk* ABTS-351 it was considered that it is unlikely that the bacterial spores will reach groundwater and therefore, no PEDGW value is required.

For the crystal proteins, during the EU evaluation of the active substance, Predicted Environmental Calculations were performed using the FOCUS PEARL 4.4.4 model in accordance with FOCUS guidance; although this model is designed for organic compounds and therefore, not strictly applicable for crystalline proteins (ẟ-endotoxins). For input to groundwater models, it was agreed during the expert meeting in March 2020, that groundwater modelling should use the following endpoints for the evaluation of crystal proteins in groundwater: soil DT50 = 41.3 days, KOC = 1000 mL/g, and a Freundlich Exponent (1/n) = 1. The groundwater exposure assessment concluded that the potential for leaching of the crystal proteins to groundwater above the parametric drinking water limit of 0.1 µg/L is low for the representative uses assessed using the FOCUS groundwater scenarios and all PECGW values following application to vegetable crops were <0.001 µg/L (EFSA, 2021).

Predicted Environmental Concentrations in groundwater (PECGW) of the crystalline proteins associated with *Btk* have been calculated for the critical GAP detailed in Table 9-1 in the report of Padricello and Tallentire (2023). The PECGW values are presented in Table 9.3.1-4 and representative the worst-case GAP for Foray® 76B to cover the risk envelope for all uses. Simulations performed have considered a maximum amount of crystal protein of 12.7% (w/w) (See this Registration Report, Part C).

|  |  |
| --- | --- |
| Data point addressed | IIIM 9.3.1/01 |
| Author(s) (year) | Padricello, V. and Tallentire, E. (2023) |
| Title | Foray® 76B: Predicted environmental concentrations in groundwater of the crystal proteins associated with *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 following application to forest and tree crops within the EU |
| Report number | Report reference 1810360.UK0-7808 |
| Test facility | - |
| Published | No |
| Test guideline | FOCUS (2021) |
| Deviations | No |
| GLP | No. Not relevant to simulation modelling |

Predicted environmental concentrations in groundwater (PECGW) were determined for the crystalline proteins associated with *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 using the FOCUS PEARL 5.5.5 and FOCUS PELMO 6.6.4 models. The GAP considered various types of perennial tall plants, including deciduous and coniferous forest, pine trees, ornamental trees and shrubs or amenity areas (parks, gardens) and therefore, the FOCUS groundwater crop apples was used as a surrogate crop. This crop group was selected to represent the entire range of forest/tree crops within the GAP. Simulations assumed a maximum amount of crystal protein content of 12.7% (w/w) of the MPCA, which was applied directly to the soil, assuming no plant interception as a worst-case scenario. The modelling simulated 26 years of continuous cropping with annual application of the MPCA, covering the period when larvae/caterpillars are present, from early season (March) to late season (October). Therefore, absolute application dates starting at the 1st day of each month were input (see Table 9.3.1-2).

A summary of the agronomic input data and the application dates are shown in the following tables. The representative FOCUS crop ‘apple’ was selected to cover the risk envelope for uses to all forest, tree and shrub vegetation detailed in the GAP. The application rate used for the groundwater simulations represents a worst-case scenario with regard to the maximum number of applications, the highest rate of application, and the minimum interval between applications.

**Table 9.3.1-1 Worst-case GAP for Foray® 76B**

| **Use** | **FOCUS crop or surrogate** | **Growth stage [BBCH]** | **Application rate of formulated product [L/ha]** | **Application rate of Active substance [g/ha]** | **Application rate of Cry Protein \* [g/ha]** | **Interval [d]** | **Crop interception** **\*\* [%]** | **Effective rate to soil of Cry Protein [g/ha]** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| All Uses (1 to 4b) | Apples\*\*\* | From BBCH 09 | 4 × 2.5 | 4 × 520 | 4 × 66.04 | 5 | 0 | 4 × 66.04 |

\* Crystal protein content is 12.7% (w/w) of the MPCA

\*\* Worst-case scenario

\*\*\* The crop apples has been used as a surrogate for forest and tree crops and to cover the risk envelope for all uses.

**Table 9.3.1-2 Application dates used for the groundwater exposure modelling with PEARL and PELMO**

| **Scenario / Crop** | **Application dates modelled** | | | |
| --- | --- | --- | --- | --- |
| **Application 1** | **Application 2** | **Application 3** | **Application 4** |
| **Forest/Tree Crops (surrogate crop Apples)** | | | | |
| Châteaudun | 1st  Mar/Apr/May/  Jun/Jul/Aug/Sep/Oct | 6th  Mar/Apr/May/  Jun/Jul/Aug/Sep/Oct | 11th  Mar/Apr/May/  Jun/Jul/Aug/Sep/Oct | 16th  Mar/Apr/May/  Jun/Jul/Aug/Sep/Oct |
| Hamburg | 1st  Mar/Apr/May/  Jun/Jul/Aug/Sep/Oct | 6th  Mar/Apr/May/  Jun/Jul/Aug/Sep/Oct | 11th  Mar/Apr/May/  Jun/Jul/Aug/Sep/Oct | 16th  Mar/Apr/May/  Jun/Jul/Aug/Sep/Oct |
| Jokioinen | 1st  Mar/Apr/May/  Jun/Jul/Aug/Sep/Oct | 6th  Mar/Apr/May/  Jun/Jul/Aug/Sep/Oct | 11th  Mar/Apr/May/  Jun/Jul/Aug/Sep/Oct | 16th  Mar/Apr/May/  Jun/Jul/Aug/Sep/Oct |
| Kremsmünster | 1st  Mar/Apr/May/  Jun/Jul/Aug/Sep/Oct | 6th  Mar/Apr/May/  Jun/Jul/Aug/Sep/Oct | 11th  Mar/Apr/May/  Jun/Jul/Aug/Sep/Oct | 16th  Mar/Apr/May/  Jun/Jul/Aug/Sep/Oct |
| Okehampton | 1st  Mar/Apr/May/  Jun/Jul/Aug/Sep/Oct | 6th  Mar/Apr/May/  Jun/Jul/Aug/Sep/Oct | 11th  Mar/Apr/May/  Jun/Jul/Aug/Sep/Oct | 16th  Mar/Apr/May/  Jun/Jul/Aug/Sep/Oct |
| Porto | 1st  Mar/Apr/May/  Jun/Jul/Aug/Sep/Oct | 6th  Mar/Apr/May/  Jun/Jul/Aug/Sep/Oct | 11th  Mar/Apr/May/  Jun/Jul/Aug/Sep/Oct | 16th  Mar/Apr/May/  Jun/Jul/Aug/Sep/Oct |
| Piacenza | 1st  Mar/Apr/May/  Jun/Jul/Aug/Sep/Oct | 6th  Mar/Apr/May/  Jun/Jul/Aug/Sep/Oct | 11th  Mar/Apr/May/  Jun/Jul/Aug/Sep/Oct | 16th  Mar/Apr/May/  Jun/Jul/Aug/Sep/Oct |
| Sevilla | 1st  Mar/Apr/May/  Jun/Jul/Aug/Sep/Oct | 6th  Mar/Apr/May/  Jun/Jul/Aug/Sep/Oct | 11th  Mar/Apr/May/  Jun/Jul/Aug/Sep/Oct | 16th  Mar/Apr/May/  Jun/Jul/Aug/Sep/Oct |
| Thiva | 1st  Mar/Apr/May/  Jun/Jul/Aug/Sep/Oct | 6th  Mar/Apr/May/  Jun/Jul/Aug/Sep/Oct | 11th  Mar/Apr/May/  Jun/Jul/Aug/Sep/Oct | 16th  Mar/Apr/May/  Jun/Jul/Aug/Sep/Oct |

The substance specific input data used in the modelling was taken from EFSA (2021) or from the RAR (2020) and is presented in Table 9.3.1-3.

**Table 9.3.1-3 Substance specific input parameters used in the FOCUS modelling for the Crystal protein**

| **Endpoint** | **Parameter value** | **Remarks** |
| --- | --- | --- |
| **Physico-chemical parameters** | | |
| Chemical name | Cry protein |  |
| Molecular weight (g/mol) | 1000 | Worst-case assumption |
| Vapour pressure (Pa) | 1.0 x 10-9 (20°C) | Worst-case assumption |
| Vapour pressure (Pa) \* | 4.0 x 10-9 (30°C) \* | PELMO input |
| Molar enthalpy of vaporization (kJ/mol) | 95 | FOCUS recommendation |
| Water solubility (mg/L) | 1000 | Worst-case assumption |
| Water solubility (mg/L) \* | 2000 \* | PELMO input |
| Molar enthalpy of dissolution (kJ/mol) | 27 | FOCUS recommendation |
| Diffusion coefficient in water  (m²/d) | 4.3 x 10-5 (20°C) (PEARL) | FOCUS recommendation |
| Diffusion coefficient in air (m²/d) | 0.43 (20°C) | FOCUS recommendation |
| **Degradation in soil** | | |
| DT50 in soil (days) | 41.3 (20°C) | EFSA (2021) |
| Transformation rate (*Btk* to Sink) | 0.0167632 | PELMO input, ln 2 / DT50 |
| Temperature correction function: - reference temperature (°C) - molar activation energy (kJ/mol) | 20 65.4 | FOCUS recommendation |
| **Sorption to soil** | | |
| Soil adsorption coefficient, KOC (mL/g) | 1000(pH independent PEARL) | EFSA (2021) |
| KOM (mL/g) | 580 | KOC /1.274 |
| Molar enthalpy of sorption (kJ/mol) | 0 | FOCUS recommendation |
| Freundlich exponent 1/n (-) | 1.0 | FOCUS default |
| Reference concentration in liquid phase (mg/L) | 1 | FOCUS recommendation |
| Desorption rate coefficient (d-1) | 0 | FOCUS recommendation |
| Factor relating CofFreNeq and CofFreEql (-) | 0 | FOCUS recommendation |
| **Crop/management related parameters** | | |
| Plant uptake factor | 0 | Worst -case |
| Application depth (cm) | 0 | FOCUS recommendation |

\*As recommended by the FOCUS PELMO manual version 5.0, which states “If the temperature dependency is not known, it is recommended to consider a factor of 4 for an increase of 10°C for the vapour pressure and a factor of 2 for the water solubility and the same temperature increase.”

PECGW values for the crystal proteins are presented in Table 9.3.1-4.

**Table 9.3.1-4 Predicted groundwater concentrations of the crystal protein following application of Foray® 76B to forests and trees (using the FOCUS crop apples as a surrogate) using FOCUS PEARL 5.5.5 and PELMO 6.6.4**

| **FOCUS Model** | **Scenario** | **Maximum 80th percentile annual average concentration (µg/L)** | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **March** | **April** | **May** | **June** | **July** | **August** | **September** | **October** |
| PEARL | Châteaudun | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Hamburg | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Jokioinen | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Kremsmünster | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Okehampton | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Porto | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Piacenza | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Sevilla | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Thiva | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| PELMO | Châteaudun | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Hamburg | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Jokioinen | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Kremsmünster | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Okehampton | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Porto | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Piacenza | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Sevilla | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Thiva | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

**Summary**

Leaching of the microorganism is not expected since its mobility is limited.

With regard to the crystal proteins associated with *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351, the PECGW values calculated using the models FOCUS PEARL 5.5.5 and FOCUS PELMO 6.6.4 were < 0.001 µg/L in all scenarios for all crops. This demonstrates an acceptable risk to groundwater from the crystal proteins contained within the formulated product, following applications made in accordance with the GAP.

## IIIM 9.4 Fate and behaviour in air

*Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 is not persistent in air and therefore, no endpoints for air are detailed in the EFSA Conclusion (2021) and no studies were performed or required during the EU evaluation of the active substance nor are required for product authorisation. The EFSA Journal 2021; 19(10):6879 details that re-aerolisation of the applied spores may occur but spore transport distances have been shown to be limited as spores rapidly lost viability following release to air. Therefore, fate and transport via air after application is unlikely to play a role in the environmental exposure to *B. thuringiensis* subsp. *kurstaki* strain ABTS-351 spores and endotoxins.

## Appendix 1: List of data submitted in support of the evaluation

| **Annex point** | **Author** | **Year** | **Title**  **Source (where different from company)**  **Company, Report No.**  **GLP or GEP status (where relevant)**  **Published or Unpublished** | **Data protection claimed Y/N** | **Owner** |
| --- | --- | --- | --- | --- | --- |
| IIIM 9.2.1/01 | Zetzmann, M. | 2023 | DiPel Biological Insecticide Technical Powder: Survivability in Surface Water  Eurofins Aquatic Ecotoxicology GmbH, Germany  Report No.: S22-04102  GLP: Yes  Unpublished | Y | XXXX |
| IIIM 9.2.1/02 | Woodhouse, J.N, Kinsela, A.S, Collins, R.N, Bowling, L.C., Honeyman, G.L., Holliday, J.K., and Neilan, B.A. | 2016 | Microbial communities reflect temporal changes in cyanobacterial composition in a shallow ephemeral freshwater lake  The ISME Journal (2016) 10, p. 1337-1351  GLP: No  Published | N | Open Literature |
| IIIM 9.2.1/03 | Bergauer, K., Fernandez-Guerra, A., Garcia, J.A.L., Sprenger, R.R., Stepanauskas, R., Pachiadaki, M.G., Jensen, O.N., and Herndl, G. J. | 2017 | Organic matter processing by microbial communities throughout the Atlantic water column as revealed by metaproteomics  Proc Natl Acad Sci U S A. (2017) 115(3), p. 445-447  GLP: No  Published | N | Open Literature |
| IIIM 9.2.1/04 | Zhou, J.; Richlen, M.L., Sehein, T.R., Kulis, D.M., Anderson, D.M., and Cai, Z. | 2018 | Microbial Community Structure and Associations During a Marine Dinoflagellate Bloom  Front. Microbiol. (2018), 9(1201), p. 1-21  GLP: No  Published | N | Open Literature |
| IIIM 9.2.1/05 | Bizic-Ionescu B., Ionescu D., and Grossart, H. | 2018 | Organic Particles: Heterogeneous Hubs for Microbial Interactions in Aquatic Ecosystems  Front. Microbiol. (2018), 9(2569), p. 1-15  GLP: No  Published | N | Open Literature |
| IIIM 9.3.1/01 | Padricello, V. and Tallentire, E. | 2023 | Foray® 76B: Predicted environmental concentrations in groundwater of the crystal proteins associated with *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 following application to forest and tree crops within the EU. Exponent Report No.: 1810360.UK0-7808.  GLP: No (modelling report)  Unpublished | N | XXXX |

## Appendix 2: GAP table

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **1** | **2** | **3** | **4** | **5** | **6** | | **7** | **8** | **9** | **10** | **11** | | **12** | **13** | **14** |
| **Use-No.** | **Member state(s)** | **Crop and/  or situation    (crop destination / purpose of crop)** | **F, Fn, Fpn  G, Gn, Gpn  or  I** | **Pests or Group of pests controlled    (additionally: developmental stages of the pest or pest group)** | **Application** | | | | | **Application rate** | | | | **PHI  (days)** | **Remarks:     e.g. g safener/synergist per ha** |
| Method / Kind | Timing / Growth stage of crop & season | | Max. number  a) per use  b) per crop/ season | Min. interval between applications (days) | kg or L product / ha  a) min / max. rate per appl.  b) max. total rate per crop/season | | g or kg as/ha   a) min / max. rate per appl.  b) max. total rate per crop/season | Water L/ha    min / max |
| 1 | IT | Coniferous and deciduous forest and green areas (trees and shrubs in parks and gardens) | F | Lepidoptera caterpillars L1 to L4  *Choristoneura sp*. - CHONSP,  *Geometridae* - 1GEOMF,  *Hyphantria cunea* - HYPHCU  *Malacosoma neustria* - MALANE,  Stilpnotia salicis - LEUOSA,  *Euproctis chrysorrhoea* - EUPRCH,  *Lymantria dispar* - LYMADI,  *Lymantria monacha* - LYMAMO,  *Thaumetopoea pityocampa* - THAUPI,  *Thaumetopoea processionea* - THAUPR,  *Dendrolimus pini* - DENDPI,  *Dendrolimus superans* - DENDSU,  *Tortrix viridana* - TORTVI | Ground spray | When caterpillars are visible following egg hatch & foliage growth sufficient for deposition | | a) 1 - 4  b) 4 | 5 days | a) 2 - 2.5 L/ha  b) 10 L/ha | | a) 0.413 - 0.516 kg a.s/ha  b) 2.06 kg a.s./ha | 0 - 500 L/ha | - | Application rate in CFU:  a) 3.02 - 3.77 x 1013 CFU/ha  b) 1.51 x 1014 CFU/ha  Aerial application only by emergency permits (Avio). |

| **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | | **12** | **13** | **14** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Use-No.** | **Member state(s)** | **Crop and/  or situation    (crop destination / purpose of crop)** | **F, Fn, Fpn  G, Gn, Gpn  or  I** | **Pests or Group of pests controlled    (additionally: developmental stages of the pest or pest group)** | **Application** | | | | **Application rate** | | | | **PHI  (days)** | **Remarks:     e.g. g safener/synergist per ha** |
| Method / Kind | Timing / Growth stage of crop & season | Max. number  a) per use  b) per crop/ season | Min. interval between applications (days) | kg or L product / ha  a) min / max. rate per appl.  b) max. total rate per crop/season | | g or kg as/ha   a) min / max. rate per appl.  b) max. total rate per crop/season | Water L/ha    min / max |
| 2 | ES | Coniferous forest, Deciduous forest, Palm trees, shurbs and small ornamental trees | F | Lepidoptera caterpillars L1 to L4  Procesionaria, *Thaumetopoea processionea* - THAUPR  Procesionaria del pino, *Thaumetopoea pityocampa* - THAUPI  Lagarta, *Lymantria spp*. - LYMASP  Oruga del zurrón, *Euproctis chrysorrhoea* - EUPRCH  Tortrix, *Tortrix viridana* - TORTVI | Spray (ground and aerial application) | When caterpillars are visible following egg hatch & foliage growth sufficient for deposition | a) 1 - 4  b) 4 | 5 days | a) 2 - 2.5 L/ha  b) 10 L/ha | | a) 0.413 - 0.516 kg a.s/ha  b) 2.06 kg a.s./ha | Aerial application: no dillution  Ground application: 0 - 500 L/ha | - | Application rate in CFU:  a) 3.02 - 3.77 x 1013 CFU/ha  b) 1.51 x 1014 CFU/ha |
| 3 | ES | Coniferous forest, Deciduous forest, Palm trees, , shurbs and small ornamental trees | F | Lepidoptera caterpillars L1 to L4 | Spray (ground and aerial application) | When caterpillars are visible following egg hatch & foliage growth sufficient for deposition | a) 1 - 4  b) 4 | 5 days | a) 1.5 - 2.5 L/ha  b) 10 L/ha | | a) 0.31 - 0.52 kg a.s/ha  b) 2.06 kg a.s./ha | Aerial application: no dillution  Ground application: 0 - 500 L/ha | - | Application rate in CFU:  a) 2.26 - 3.77 x 1013 CFU/ha  b) 1.51 x 1014 CFU/ha |
| 4 | LT | Forest | F | Lepidoptera caterpillars  *Lymantria monacha* - LYMAMO  *Dendrolimus pini* - DENDPI  *Tortrix viridana* - TORTVI | Spray | When caterpillars are visible following egg hatch & foliage growth sufficient for deposition | a) 2  b) 2 | 7 days | a) 2 - 2.5 L/ha  b) 5 L/ha | | a) 0.413 - 0.516 kg a.s/ha  b) 1.03 kg a.s./ha | - | - | Application rate in CFU:  a) 3.02 - 3.77 x 1013 CFU/ha  b) 1.51 x 1014 CFU/ha |

| **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Use-No.** | **Member state(s)** | **Crop and/  or situation    (crop destination / purpose of crop)** | **F, Fn, Fpn  G, Gn, Gpn  or  I** | **Pests or Group of pests controlled    (additionally: developmental stages of the pest or pest group)** | **Application** | | | | **Application rate** | | | **PHI  (days)** | **Remarks:     e.g. g safener/synergist per ha** |
| Method / Kind | Timing / Growth stage of crop & season | Max. number  a) per use  b) per crop/ season | Min. interval between applications (days) | kg or L product / ha  a) min / max. rate per appl.  b) max. total rate per crop/season | g or kg as/ha   a) min / max. rate per appl.  b) max. total rate per crop/season | Water L/ha    min / max |
| 5 | PL | Pine trees | F | *Lymantria monacha* - LYMAMO  *Dendrolimus pini* - DENDPI | Spray | When caterpillars are visible following egg hatch & foliage growth sufficient for deposition | a) 1 - 4  b) 4 | 5 days | a) 2.5 L/ha  b) 10 L/ha | a) 0.52 kg a.s/ha  b) 2.06 kg a.s./ha | - | - | Application rate in CFU:  a) 3.77 x 1013 CFU/ha  b) 1.51 x 1014 CFU/ha |
| 6 | PL | Deciduous forest | F | *Operophtera brumata* - CHEIBR  *Tortrix viridana* - TORTVI | Spray | When caterpillars are visible following egg hatch & foliage growth sufficient for deposition | a) 1 - 4  b) 4 | 5 days | a) 2.5 L/ha  b) 10 L/ha | a) 0.52 kg a.s/ha  b) 2.06 kg a.s./ha | UVL application: 0-10 L/ha,  application of high pressure (10 bar): 200 L/ha,  application of low pressure (2-3 bar): 600 L/ha. | - | Application rate in CFU:  a) 3.77 x 1013 CFU/ha  b) 1.51 x 1014 CFU/ha |
| 7 | PL | Deciduous forest | F | *Euproctis chrysorrhoea* - EUPRCH | Spray | When caterpillars are visible following egg hatch & foliage growth sufficient for deposition | a) 1 - 2  b) 2 | 14 days | a) 3 L/ha  b) 6 L/ha | a) 0.619 kg a.s/ha  b) 1.24 kg a.s./ha | UVL application: 0-10 L/ha,  application of high pressure (10 bar): 200 L/ha,  application of low pressure (2-3 bar): 600 L/ha. | - | Application rate in CFU:  a) 4.53 x 1013 CFU/ha  b) 9.06 x 1013 CFU/ha |

| **1** | **2** | | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Use-No.** | | **Member state(s)** | **Crop and/  or situation    (crop destination / purpose of crop)** | **F, Fn, Fpn  G, Gn, Gpn  or  I** | **Pests or Group of pests controlled    (additionally: developmental stages of the pest or pest group)** | **Application** | | | | **Application rate** | | | **PHI  (days)** | **Remarks:     e.g. g safener/synergist per ha** |
| Method / Kind | Timing / Growth stage of crop & season | Max. number  a) per use  b) per crop/ season | Min. interval between applications (days) | kg or L product / ha  a) min / max. rate per appl.  b) max. total rate per crop/season | g or kg as/ha   a) min / max. rate per appl.  b) max. total rate per crop/season | Water L/ha    min / max |
| 8 | | RO | Coniferous forest | F | Lepidoptera caterpillars L1 to L4  *Choristoneura spp.* - CHONSP  *Lymantria monacha* - LYMAMO  *Thaumetopoea pityocampa* - THAUPI  *Dendrolimus pini* - DENDPI | Spray | When caterpillars are visible following egg hatch & foliage growth sufficient for deposition | a) 4 b) 4 | 5 days | a) 2 - 2.5 L/ha  b) 10 L/ha | a) 0.413 - 0.516 kg a.s/ha b) 2.06 kg a.s./ha | 0-10 L/ha (undiluted for ULV application) | - | Application rate in CFU:  a) 3.02 - 3.77 x 1013 CFU/ha  b) 1.51 x 1014 CFU/ha |
| 9 | | RO | Deciduous forest | F | Lepidoptera caterpillars L1 to L4  *Hyphantria cunea* - HYPHCU  *Malacosoma neustria* - MALANE  *Stilpnotia salicis* - LEUOSA  *Euproctis chrysorrhoea* - EUPRCH  *Lymantria dispar* - LYMADI  *Thaumetopoea processionea* - THAUPR  *Tortrix viridana* - TORTVI  *Operophtera bru*mata - CHEIBR | Spray | When caterpillars are visible following egg hatch & foliage growth sufficient for deposition | a) 4 b) 4 | 5 days | a) 2 - 2.5 L/ha  b) 10 L/ha | a) 0.413 - 0.516 kg a.s/ha b) 2.06 kg a.s./ha | 0-600 L/ha (undiluted for ULV application; high pressure application: 200L/ha and low pressure application 600 L/ha) | - | Application rate in CFU:  a) 3.02 - 3.77 x 1013 CFU/ha  b) 1.51 x 1014 CFU/ha |

| **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Use-No.** | **Member state(s)** | **Crop and/  or situation    (crop destination / purpose of crop)** | **F, Fn, Fpn  G, Gn, Gpn  or  I** | **Pests or Group of pests controlled    (additionally: developmental stages of the pest or pest group)** | **Application** | | | | **Application rate** | | | **PHI  (days)** | **Remarks:     e.g. g safener/synergist per ha** |
| Method / Kind | Timing / Growth stage of crop & season | Max. number  a) per use  b) per crop/ season | Min. interval between applications (days) | kg or L product / ha  a) min / max. rate per appl.  b) max. total rate per crop/season | g or kg as/ha   a) min / max. rate per appl.  b) max. total rate per crop/season | Water L/ha    min / max |
| 10 | HU | Deciduous forest species  (also on public areas) | F | Lepidopteran foliage pests  *Lymantria dispar* - LYMADI  *Hyphantria cunea* - HYPHCU  *Euproctis chrysorrhoea* - EUPRCH  *Aporia crataegi* - APORCR  *Thaumetopoea processionea* - THAUPR  *Tortrix viridana* - TORTVI  *Geometridae -* 1GEOMF  *Tortricidae* - 1TORTF  *Gracillariidae* - 1GRACF | Spray | When caterpillars are visible following egg hatch & foliage growth sufficient for deposition | a) 4 b) 4 | 5 days | a) 2 - 2.5 L/ha  b) 10 L/ha | a) 0.413 - 0.516 kg a.s/ha b) 2.06 kg a.s./ha | Ground spray: 600 - 1500 L/ha  Aerial spray: 60 - 80 L/ha | - | Application rate in CFU:  a) 3.02 - 3.77 x 1013 CFU/ha  b) 1.51 x 1014 CFU/ha |
| 11 | HU | Pine species  (also on public areas) | F | Lepidopteran foliage pests  *Dendrolimus pini -* DENDPI *Rhyacionia buoliana* - EVETBU *Gracillariidae* - 1GRACF | Spray | When caterpillars are  visible following egg hatch & foliage growth sufficient for deposition | a) 4 b) 4 | 5 days | a) 2 - 2.5 L/ha b) 10 L/ha | a) 0.413 - 0.516 kg a.s/ha b) 2.06 kg a.s./ha | Ground spray: 600 - 1500 L/ha  Aerial spray: 60 - 80 L/ha | - | Application rate in CFU:  a) 3.02 - 3.77 x 1013 CFU/ha  b) 1.51 x 1014 CFU/ha |

| **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Use-No.** | **Member state(s)** | **Crop and/  or situation    (crop destination / purpose of crop)** | **F, Fn, Fpn  G, Gn, Gpn  or  I** | **Pests or Group of pests controlled    (additionally: developmental stages of the pest or pest group)** | **Application** | | | | **Application rate** | | | **PHI  (days)** | **Remarks:     e.g. g safener/synergist per ha** |
| Method / Kind | Timing / Growth stage of crop & season | Max. number  a) per use  b) per crop/ season | Min. interval between applications (days) | kg or L product / ha  a) min / max. rate per appl.  b) max. total rate per crop/season | g or kg as/ha   a) min / max. rate per appl.  b) max. total rate per crop/season | Water L/ha    min / max |
| 12 | HU | Ornamental trees, bushes  (also on public areas) | F | Lepidopteran foliage pests  *Lymantria dispar* - LYMADI *Hyphantria cunea* - HYPHCU *Euproctis chrysorrhoea* - EUPRCH *Aporia crataegi* - APORCR *Thaumetopoea processionea* - THAUPR *Tortrix viridana* - TORTVI *Geometridae* - 1GEOMF *Tortricidae*  - 1TORTF *Gracillariidae -* 1GRACF *Dendrolimus pini* - DENDPI *Rhyacionia buoliana -* EVETBU | Spray | When caterpillars are  visible following egg hatch & foliage growth sufficient for deposition | a) 4 b) 4 | 5 days | a) 2 - 2.5 L/ha b) 10 L/ha | a) 0.413 - 0.516 kg a.s/ha b) 2.06 kg a.s./ha | Ground spray: 600 - 1200 L/ha  Aerial spray: 60 - 80 L/ha | - | Application rate in CFU:  a) 3.02 - 3.77 x 1013 CFU/ha  b) 1.51 x 1014 CFU/ha |
| 13 | DE | Coniferous forest, Deciduous forest | F | Lepidoptera caterpillars  L1 to L3 | Ground spray | When caterpillars are visible following egg hatch & foliage growth sufficient for deposition | a) 2  b) 2 | 14 days | a) 2 - 2.5 L/ha  b) 5 L/ha | a) 0.413 - 0.516 kg a.s/ha  b) 1.03 kg a.s./ha | 600 L/ha | - | Application rate in CFU:  a) 3.02 - 3.77 x 1013 CFU/ha  b) 1.51 x 1014 CFU/ha |
| 14 | DE | Coniferous forest, Deciduous forest | F | Lepidoptera caterpillars  L1 to L3 | Aerial spray | When caterpillars are visible following egg hatch & foliage growth sufficient for deposition | a) 2  b) 2 | 30 days | a) 2 - 2.5 L/ha  b) 5 L/ha | a) 0.516 kg a.s/ha  b) 1.03 kg a.s./ha | 70 L/ha | - | Application rate in CFU:  a) 3.77 x 1013 CFU/ha  b) 1.51 x 1014 CFU/ha |
| 15 | DE | Ornamental trees | F | Lepidoptera caterpillars  L1 to L3 | Ground spray | When caterpillars are visible following egg hatch & foliage growth sufficient for deposition | a) 1  b) 1 | NA | a) 2 - 2.5 L/ha  b) 5 L/ha | a) 0.413 - 0.516 kg a.s/ha  b) 0.516 kg a.s/ha | 600 L/ha | - | Application rate in CFU:  a) 3.02 - 3.77 x 1013 CFU/ha  b) 3.77 x 1013 CFU/ha |

|  |  |  |  |
| --- | --- | --- | --- |
| **Remarks**  **columns:** | 1 Numeration necessary to allow references  2 Use official codes/nomenclatures of EU Member States  3 For crops, the EU and Codex classifications (both) should be used; when relevant, the use situation should be described (e.g. fumigation of a structure)  4 F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application  5 Scientific names and EPPO-Codes of target pests/diseases/ weeds or, when relevant, the common names of the pest groups (e.g. biting and sucking insects, soil born insects, foliar fungi, weeds) and the developmental stages of the pests and pest groups at the moment of application must be named.  6 Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench  Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated. |  | 7 Growth stage at first and last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 38263-3152-4), including where relevant, information on season at time of application  8 The maximum number of application possible under practical conditions of use must be provided.  9 Minimum interval (in days) between applications of the same product  10 For specific uses other specifications might be possible, e.g.: g/m³ in case of fumigation of empty rooms. See also EPPO-Guideline PP 1/239 Dose expression for plant protection products.  11 The dimension (g, kg) must be clearly specified. (Maximum) dose of a.s. per treatment (usually g, kg or L product / ha).  12 If water volume range depends on application equipments (e.g. ULVA or LVA) it should be mentioned under “application: method/kind”.  13 PHI - minimum pre-harvest interval  14 Remarks may include: Extent of use/economic importance/restrictions |

## Appendix 3: Additional Information

The PEC/PEDSW values presented in the EFSA Journal 2021; 19(10):6879 consider both a ditch volume of 210 L/m2 and 300 L/m2, respectively. A ditch of 210 L/m2 is based upon the Netherlands National Surface Water Model TOXSWA v1.2 where the ditch has sloping sides (Beltman and Adriaanse 1999[[6]](#footnote-7)), whereas a volume of 300 L/m2 is based upon the FOCUS dimensions for a rectangular 1 m section of ditch which is 1 m wide and 0.3 m deep. The FOCUS dimensions of the ditch are detailed in the FOCUS surface water guidance and are used for FOCUS Step 1-2 calculations, which consider a volume of 300 L/m2. Following the methodology presented in the EFSA Journal, the two sets of calculations have been performed; however, the PEC/PEDSW values calculated for a ditch with a volume of 210 L/m2 are for the Netherlands only. Therefore, they are presented here for information only alongside the calculations based on a volume of 300 L/m2 that are relevant for all other EU MS.

**Table A3-1 PEDSW and PECSW values for the spray drift pathway (using the FOCUS / Rautmann Drift values)**

| **Use** | **Drift Crop** | **Rate of Application** | **Drift values [%]** | **Ditch volumetry\*** | | **PECsw (single application)** | **PECsw (multiple applications)** | **Unit** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Formulated product (PECSW)** | | | | | | | | |
| All Uses | Aerial | 1 × 3360 g Foray® 76B / ha | 33.2 | *210 L/m2\** | | *531.20* | *-* | *µg/L* |
| 300 L/m2 | | 371.84 | - | µg/L |
| Fruit trees, early application\*\*\* | 1 × 3360 g Foray® 76B / ha | 29.2 | *210 L/m2\** | | *467.20* | *-* | *µg/L* |
| 300 L/m2 | | 327.04 | - | µg/L |
|  | **Active Substance (PECSW)** | | | | | | | | |
| All Uses | Aerial | 4 × 520 g MPCA/ha  (1 × 619 g MPCA/ha) | 33.2 / 33.2 | *210 L/m2\** | | *97.86* | *328.84* | *µg/L* |
| 300 L/m2 | | 68.50 | 230.19 | µg/L |
| Fruit trees, early application\*\*\* | 4 × 520 g MPCA/ha  (1 × 619 g MPCA/ha) | 29.20 / 23.61 | *210 L/m2\** | | *86.07* | *233.85* | *µg/L* |
| 300 L/m2 | | 60.25 | 163.70 | µg/L |
|  | **Active Substance (in Colony Forming Units; CFU; PEDSW)** | | | | | | | | |
| All Uses | Aerial | 4 × 4.23 × 1013 CFU/ha  (1 × 5.07 × 1013 CFU/ha) | 33.2 / 33.2 | *210 L/m2\** | *8.02 × 106* | | *2.67 × 107* | *CFU/L* |
| 300 L/m2 | 5.61 × 106 | | 1.87 × 107 | CFU/L |
| Fruit trees, early application\*\*\* | 4 × 4.23 × 1013 CFU/ha  (1 × 5.07 × 1013 CFU/ha) | 29.20 / 23.61 | *210 L/m2\** | *7.05 × 106* | | *1.90 × 107* | *CFU/L* |
| 300 L/m2 | 4.93 × 106 | | 1.33 × 107 | CFU/L |
|  | **Cry proteins (PECSW)\*\*** | | | | | | | | |
| All Uses | Aerial | 4 × 66.04 g CryP/ha  (1 × 78.61 g CryP/ha) | 33.2 / 33.2 | *210 L/m2\** | *12.43* | | *35.02\* (41.76)* | *µg/L* |
| 300 L/m2 | 8.70 | | 24.51\* (29.23) | µg/L |
| Fruit trees, early application\*\*\* | 4 × 66.04 g CryP/ha  (1 × 78.61 g CryP/ha) | 29.20 / 23.61 | *210 L/m2\** | *10.93* | | *24.90\* (29.70)* | *µg/L* |
| 300 L/m2 | 7.65 | | 17.43\* (20.79) | µg/L |

\* Values presented for information only (only relevant to the NL ditch).

\*\* PECsw (spray drift pathway) considering degradation in between 4 applications (DT50 = 28 days). In brackets, PECsw not taking into account the degradation in between applications (as worst-case). Total seasonal dose was used.

\*\*\* Drift values for pome/stone fruit trees (early application) used as a surrogate for application to trees and forest plantations; these drift values are worst-case for shrubs.

1. OECD (2012); OECD Guidance ot the Environmental Safety Evaluation of Microbial Biocontrol Agents. Series on Pesticides No. 67. Reference ENV/JM/MONO(2012)1. 17 Feb 2012. [↑](#footnote-ref-2)
2. EFSA (2014). EFSA Guidance Document for evaluating laboratory and field dissipation studies to obtain DegT50 values of active substances of plant protection products and transformation products of these active substances in soil. EFSA Journal 2014; 12(5):3662. [↑](#footnote-ref-3)
3. Rautmann, D., Streloke, M. and Winkler, R. (2001): New basic drift values in the authorisation procedure for plant protection products. In: Workshop on Risk Assessment and Risk Mitigation Measures in the context of the Authorisation of Plant Protection Products (WORMM; R. Forster., Streloke, M. Eds), 27-29 September, 1999, Heft 383, BBA, Berlin and Braunschweig, Germany. [↑](#footnote-ref-4)
4. FOCUS (2015): Generic guidance for FOCUS surface water Scenarios v 1.4, May 2015. [↑](#footnote-ref-5)
5. Beltman W.H.J. and Adriaanse P.I. (1999): User’s manual TOXSWA 1.2. Simulation of pesticide fate in small surface waters. Wageningen, Win and Staring Centre. Technical Document 54. DOC. 112 p.; 44 Figs; 3 Tables; 36 Refs., 2 Annexes [↑](#footnote-ref-6)
6. Beltman W.H.J. and Adriaanse P.I. (1999): User’s manual TOXSWA 1.2. Simulation of pesticide fate in small surface waters. Wageningen, Win and Staring Centre. Technical Document 54. DOC. 112 p.; 44 Figs; 3 Tables; 36 Refs., 2 Annexes [↑](#footnote-ref-7)