Regulation (EU) No 528/2012 concerning the making available on the market and use of biocidal products

**PRODUCT ASSESSMENT REPORT OF A BIOCIDAL PRODUCT FOR NATIONAL AUTHORISATION APPLICATIONS**

(submitted by the evaluating Competent Authority)



AQUABAC DF 3000

Product type 18

*Bacillus thuringiensis,* subsp. *Israelensis* BMP 144

NA-APP Case Number in R4BP: BC-SV010815-11

Evaluating Competent Authority: France

Date: August 2019

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# History of the dossier

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| --- | --- | --- | --- | --- |
| **Application type** | **refMS** | **Case number in the refMS** | **Decision date** | **Assessment carried out (i.e. first authorisation / amendment /renewal)** |
| NA-APP | *FR* | *BC-SV010815-11* | 30/06/2016 | Initial assessment : AQUABAC DF3000 |
| na | *FR* | na | - | Post-authorisation data submitted in 2017 |
| na | *FR* | na | - | Post-authorisation data submitted in 2019 |
| na | *FR* | na | February 2019 | Cancellation of the authorization |

## Information on the substance(s) of concern

Considering the definition of a substance of concern set in the Guidance on the BPR Volume III Human Health – Part B Risk Assessment (updated Version 4.0, December 2017), the product AQUABAC DF 3000 contains **Sodium Dibutylnaphthalene sulphonate** (at 10%w/w) as a substance of concern (band A).

## Endocrine disrupting properties

None of the co-formulants contained in the product AQUABAC DF 3000 is identified as endocrine disruptors. Please refer to Confidential Annex.

## Documentation

### Data submitted in relation to product application

**Identity, physico-chemical and analytical method data**

The active substance *Bacillus thuringiensis subspecies israelensis* *serotype H14,* strain AM65-52 is included in the Union list of approved active substances under Directive 98/8/EC. The strain *Bacillus thuringiensis Israelensis* BMP 144 submitted by CERA SAS is considered as equivalent in term of identity to the reference source. A technical equivalence report presenting this conclusion was prepared by France.

|  |  |  |  |
| --- | --- | --- | --- |
| **Common name** | *Bti* Strain BMP 144 | | |
| **Taxonomic names** | **Species:** | | *thuringiensis* |
| **Subspecies:** | | *israelensis* |
| **Serotype:** | | H-14 |
| **Strain:** | | BMP 144 |
| **Genus:** | | *Bacillus* |
| **Family:** | | Bacillaceace |
| **Collection and culture reference number** | ATTC number: SD-6993  Designation: strain BMP 144 | |  |
| **Purity in the technical active substance (BMP144 primary powder or AQUABAC primary powder):** | **Minimum** | **Maximum** | **Nominal** |
| 100 % | 100 % | 100 % (384.4 g/kg) |
| 3.2 × 109 ITU/pound (7000 ITU/mg ) | 3.2 × 109 ITU/pound (7000 ITU/mg ) | 7000 ITU/mg |
| **Certified content of the acitive substance in the product AQUABAC DF 3000** | **Minimum** | **Maximum** | **Nominal** |
| 41.71% | 44.29% | 43.0% |
| 3000 ITU/mg | 3583 ITU/mg | 3404 ITU/mg |
| 1.54 × 1010 CFU/g | 3.74 × 1010 CFU/g | 2.48 × 1010 CFU/g |

Physico-chemical properties studies and analytical methods on the biocidal product AQUABAC DF3000 were provided by the Applicant CERA.

**Efficacy data**

* Laboratory test according to WHO[[1]](#footnote-1) method with the product AQUABAC DF 3000 (43 % w/w *Bti* BMP 144) on 3rd and early 4th larval stages of *Aedes aegyti, Aedes albopictus, Culex pipiens and Anopheles gambiae*.
* Semi-field test conducted with the product AQUABAC DF3000 (43 % w/w Bti BMP 144) according to an internal method on *Ochlerotatus* (=*Aedes) taeniorhynchus* and *Cx. quinquefasciatus*.
* Field test conducted in France with the product AQUABAC DF 3000 (43 % w/w *Bti* BMP 144) according to an internal method on *Ochlerotatus (Aedes) caspius,* using manual ground application.
* Semi-field test conducted in France with the products AQUABAC 200 G (2.86 % w/w Bti BMP 144), AQUABAC XT (8 % w/w Bti BMP 144) and AQUABAC DF3000 (43 % m/m Bti BMP 144), according to an internal method, on larval stages of *Culex quinquefasciatus*.
* Semi-field test conducted in France with the products AQUABAC XT (8 % w/w Bti BMP 144, new composition) on larval stages of *Culex quinquefasciatus* and *Aedes albopictus*, and AQUABAC DF3000 (43 % m/m Bti BMP 144) on larval stages of *Aedes albopictus*, according to an internal method.

The applicant also submitted studies coming from the literature performed with other products. These data on other products are not considered as acceptable by the eCA, above all, when product forms, application modes or application rates are not comparable. Even in case of a comparable product forms and application rates, differences in manufacturing process that could impact the toxic activity of Bti crystals after application cannot be exclude. Therefore, these studies are not taken into account in this evaluation.

**Toxicology data**

No new toxicological study was provided.

**Residue data**

No specific residue data were submitted in the context of this dossier. The product AQUABAC DF 3000 is intended to be applied by professional users, outdoor on flood water, roadside ditches, irrigation ditches, floodwater, rice fields, pastures, woodland pools, snowmelt pools, standing pools, tidal water, salt marshes, catch basins, storm water retention areas, standing water in fields growing crops( such as alfalfa, almonds, asparagus, corn, cotton, dates, grapes, peaches, and walnuts), sewage lagoons and animal waste lagoons.

No data on potential exposure were submitted. However, as regard to the use in water irrigating rice, AQUABAC DF 3000 will only be applied in presence of water when mosquitoes proliferate and towards the end of the rice growing period the fields are dried approximately 4 weeks before the grain harvest. Rice grains are also covered by a husk that is removed prior to consumption.

**Ecotoxicology data**

No new ecotoxicological study was provided.

### Access to documentation

No letter of access is submitted for data related to the product.

# Summary of the product assessment

**1. Administrative information**

**1.1. Trade name(s) of the product**

| **Trade name(s)** |  |
| --- | --- |
| AQUABAC DF3000 |  |

**1.2. Authorisation holder**

|  |  |  |
| --- | --- | --- |
| **Name and address of the authorisation holder** | **Name** | CERA SAS |
| **Address** | 16, rue de Saint-Pétersburg,  75008 PARIS  France |
| **Authorisation number** | First authorisation | |
| *R4BP asset reference number* |  | |
| **Date of the authorisation** | 19/08/2019 | |
| **Expiry date of the authorisation** | 18/08/2029 | |

**1.3. Manufacturer(s) of the product**

|  |  |
| --- | --- |
| **Name of manufacturer** | Becker Microbial Products Inc |
| **Address of manufacturer** | Becker Microbial Products Inc  11146 N.W. 69TH Place  Parkland  FL 33076  USA |
| **Location of manufacturing sites** |

**1.4. Manufacturer(s) of the active substance(s)**

|  |  |
| --- | --- |
| **Active substance** | *Bacillus thuringiensis israelensis* strain BMP 144 (Bti BMP 144)[[2]](#footnote-2) |
| **Name of manufacturer** | Becker Microbial Products Inc |
| **Address of manufacturer** | Becker Microbial Products Inc  11146 N.W. 69TH Place  Parkland  FL 33076  USA |
| **Location of manufacturing sites** |

**2. Product composition and formulation**

**2.1. Qualitative and quantitative information on the composition of the product**

| **Common name** | **Function** | **CAS number** | **EC number** | **Content (%)** |
| --- | --- | --- | --- | --- |
| *Bacillus thuringiensis,* subsp. *Israelensis* BMP 144 | Active substance | - | - | 43 %  Minimum 3000 ITU/mg |
| Sodium Dibutylnaphthalene sulphonate | Dispersant | 91078-64-7 | 293-346-9 | 10 % |

**2.2. Type of formulation**

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| Wettable granule (WG) |

**3. Hazard and precautionary statements**

| **Classification** | |
| --- | --- |
| Hazard category | Eye irritation, category 2 |
| Hazard statement | H319 Causes serious eye irritation |
|  | |
| **Labelling** | |
| Signal words | Warning |
| Hazard statements | H319 Causes serious eye irritation |
| Precautionary statements | P264 Wash … thoroughly after handling  P280 Wear protective gloves/protective clothing/eye protection/face protection  P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.  P337+P313 If eye irritation persists: Get medical advice/attention. |
|  | |
| Note | Contains *Bacillus thuringiensis israelensis*, micro-organisms may have a potential to provoke sensitising reactions. |

**4. Authorised use(s)**

**4.1. Use description**

**Table 1. Use # 1 – professionals**

|  |  |
| --- | --- |
| **Product Type** | 18 |
| **Where relevant, an exact description of the authorised use** | AQUABAC DF3000 is used for the control of mosquito larvae in water where mosquito breeding occurs |
| **Target organism(s) (including development stage)** | Mosquitoes  *Aedes* spp. and *Culex* spp.  Larvae (L1 to early L4) |
| **Field(s) of use** | Outdoor use  Surface waters |
| **Application method(s)** | Spraying  Ground application by conventional hand-held equipment  The product is to be diluted in water prior to application. |
| **Application rate(s) and frequency** | 1 kg/ha  (waters containing moderate levels of organic matter)  Time delay : within 24 - 48H after application |
| **Category(ies) of users** | Professionals |
| **Pack sizes and packaging material** | The packaging of the biocidal product AQUABAC DF3000 are HPDE jar containing 1.3 L (containing 500 g of product) and carton box (25 kg). |

***4.1.1.* *Use-specific instructions for use***

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***4.1.2. Use-specific risk mitigation measures***

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***4.1.3. Where specific to the use, the particulars of likely direct or indirect effects, first aid instructions and emergency measures to protect the environment***

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***4.1.4. Where specific to the use, the instructions for safe disposal of the product and its packaging***

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***4.1.5. Where specific to the use, the conditions of storage and shelf-life of the product under normal conditions of storage***

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**5. General directions for use**

**5.1. Instructions for use**

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| --- |
| * To ensure a satisfactory level of efficacy and avoid the development of resistance in susceptible insect populations, the following recommendations have to be implemented:   + Always read the label or leaflet before use and respect follow all the instructions provided.   + Adopt integrated pest management methods such as the combination of chemical, physical control methods and other public health measures, taking into account local specificities (climatic conditions, target species, conditions of use, etc).   + Equipment used for treatments must be appropriate, properly maintained and calibrated.   + Take into account the life cycle and characteristics of target insects to adapt treatments. In particular, target the most susceptible stage of the pest, timing of applications and areas to be treated.   + Inform the authorisation holder if the treatment is ineffective.   + Alternate products containing active substances with a different mode of action.   + Do not [use/apply] the product in areas where resistance to the active substance (s) contained in this product is supected or established.   + Check the efficacy of the product on site: if needed, causes of reduced efficacy must be investigated to ensure that there is no resistance or to identify potential resistance. * The diluted product should be applied with continuous agitation. * Before treatment, determine precisely [the areas to be treated/routes to be followed] (on a map or a plan) . * To optimise the treatment efficacy, do not apply in case of wind (above 15 km/h). * The product is not intended to be used in water water with high organic matter. * Spraying must not be carried out where there is upward air movement or where a temperature inversion prevents the spray cloud settling within the treated area. |

**5.2. Risk mitigation measures**

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| * Professionals must wear gloves, working coverall, goggles and respiratory mask (with P3 filter) during mixing/loading and application. * AQUABAC DF3000 should not be used by professional workers affected by immunodeficiency, primary or secondary, or in treatment with immunosuppressive agents, which can significantly reduce the effectiveness of the immune system response. * In case of re-entry after treatment, it is recommended to workers to wear a working coverall and gloves. * Non users are not permitted in area being treated.When used in water irrigating rice, a pre harvest interval of 1 month is required. * Do not apply in standing water in fields growing crops when edible parts of plants are present (except rice). * Do not exceed 8 applications/year with an interval of 5 days between two applications. * The labeling of the product should provide information to the user about the responsibility to follow any local requirements regarding consultation with relevant authority, before the use of AQUABAC DF3000 in a natural water habitat. * When applying AQUABAC DF3000 to ecosystems of great value for biodiversity, i.e. Natura 2000 or nature reserve, specific permission is required. * The user shall keep records of all uses, including treated areas and concentrations used, for at least 10 years and upon request provide the information to authorities or research. |

**5.3. Particulars of likely direct or indirect effects, first aid instructions and emergency measures to protect the environment**

|  |
| --- |
| * Skin contact: Remove contaminated clothing and shoes. Wash contaminated skin with water. Contact poison treatment specialist if symptoms occur. * Eye contact: Immediately flush with plenty of water, occasionally lifting the upper and lower eyelids. Check for and remove any contact lenses if easy to do. Continue to rinse with tepid water for at least 10 minutes. Get medical attention if irritation or vision impairment occurs. * Ingestion: Wash out mouth with water. Contact poison treatment specialist. Seek medical advice immediately if symptoms occur and/or large quantities have been ingested. Do not give fluids or induce vomiting. * Inhalation of dust: Remove victim to fresh air and keep at rest in a position comfortable for breathing. Seek medical advice immediately if symptoms occur and/or large quantities have been inhaled. * In case of impaired consciousness place in recovery position and seek medical advice immediately. * Keep the container or label available. |

**5.4. Instructions for safe disposal of the product and its packaging**

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| * Dispose of unused product, its packaging and all other waste in accordance with local regulations. * Do not discharge unused product on the ground, into water courses, into pipes (sink, toilets…) nor down the drains. |

**5.5. Conditions of storage and shelf-life of the product under normal conditions of storage**

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| * Shelf life: 24 months * Do not store above 20° C * Store away from light. |

**6. Other information**

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## Physico/chemical properties and analytical methods

* + 1. **Active ingredient**

#### Identity, origin of active ingredient

The source of the active substance used in the biocidal product AQUABAC DF 3000 is the different from the source included in the Union list of approved active substances.

The strain *Bacillus thuringiensis Israelensis* BMP 144 submitted by CERA SAS is considered as equivalent in term of identity to the reference source *Bacillus thuringiensis israelensis* *serotype H14,* strain AM65-52 as included into Annex I of Directive 98/8/EC. A technical equivalence report presenting this conclusion was prepared by France..

Trade name of the active substance: /

#### Physico-chemical properties

The manufacturing process is continuous. Only physical and chemical properties on the biocidal product AQUABAC DF 3000 should be evaluated. See 2.3.2.2 for physical and chemical properties of AQUABAC DF 3000.

#### Analytical method for determination of active ingredient and impurities in the technical active ingredient

An analytical method for the determination of microbial active substance *Bti* BMP144 in the AQUABAC PRIMARY POWDER was evaluated at France level in the CERA equivalence report.

The manufacturing process is continuous for the biocidal product AQUABAC XT. Methods for the determination of *Bti* BMP144 in AQUABAC XT were evaluated in the section 2.3.2.3.

#### Analytical method for determining relevant components and/or residues in different matrices

Residue analytical methods for *Bti* BMP144 are not considered necessary as no residue definition and no MRL were set for plants, food of animal origin, soil, water and air.

* + 1. **Biocidal product**
       1. **Identity, composition of the biocidal product**

The biocidal product AQUABAC DF 3000 is not the same as the one assessed for the inclusion of the active substance into annex 1 of Directive 98/8/EC. The biocidal product is a wettable granular (WG).

Trade name of the biocidal product: /

The composition of the product is confidential and is presented in a confidential annex.

The product contains a nominal content of 43 % w/w (nominal 33404 ITU/mg) of the microbial technical active substance (BMP 144 primary powder 100 %) of *Bacillus thuringiensis subsp. Israelensis* strain BMP 144.

Minimum content of the microorganism in the biocidal product: 41.71 % w/w (3000 ITU/mg)

Maximum content of the microorganism in the biocidal product: 44.29 % w/w (3583 ITU/mg)

Minimum, maximum and nominal certified concentration of the micro-organism in “BMP144” in terms of CFU/g in the product AQUABAC DF 3000:

Minimum: 1.54 × 1010 CFU/g

Nominal: 2.48 × 1010 CFU/g

Maximum: 3.74 × 1010 CFU/g

* + - 1. **Physico-chemical properties**

The tested product is AQUABACDF 3000. *Bti* Strain BMP 144’s content in tested product is 43.0 % w/w (min 3000 ITU/mg).

Type of the formulation: Wettable granule (WG)

Concentration uses proposed by the Notifier:

Minimum and maximum volumes of water per hectare for each application (aerial and terrestrial) were required but not provided by the Applicant.

According the study Brux A., 2015, it is indicated:

* Minimum use concentration of 1 % (w/v)
* Maximum use concentration of 10 %(w/v)

These concentrations were used for the evaluation of physical and chemical properties of the biocidal product AQUABAC DF 3000.

Physical and chemical properties evaluated are summarised below:

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **SECTION IIIB 2** | **PHYSICAL, CHEMICAL AND TECHNICAL PROPERTIES** | | | | | **FR evaluation** |
| **Test or study & Annex point** | **Guideline and method** | **Test material and purity specification** | **Findings and comments** | **GLP Y/N** | **Reference** |  |
| **IIIB 2.1 Appearance, colour and odour**  **(B. 3.2)** | Visual inspection and subjective assessment of odour | Aquabac 200G  Batch: 2531732  a.i. content: 3000 ITU/mg | Appearance: Granule  Colour: Light tan  Odour: Fresh acidic, fermentation | - | Certificate of Analysis (2012) | Acceptable |
| Aquabac DF 3000  Batch : 2141814 | Solid brown grain | - | Brux, A. (2015) | Acceptable |
| **IIIB 2.2 (B. 3.5)** | **Storage – stability and shelf-life** | | | | | |
| **IIIB 2.2.1 Effects of light (B. 3.5.1), temperature and humidity (B. 3.5.2) on technical characteristics of the biocidal product** | Complementary data March 2015 | Aquabac DF 3000  Batch : 2141814  CIPAC MT 46.3 | Packaging: glass   |  |  |  | | --- | --- | --- | |  | **T0** | **After 2 weeks at 54 °C** | | LC 50 (mcg/mL)  (Method: Biopotency (  Determination of the mortality of larva), see 2.3.2.3.1)  (not GLP) | 0.0570 | 0.0716 | | Appearance  SOP-PR-015 visually | Solid brown grain | **Solid brown-orange grain** | | pH at ambient temperature ( at 1 %)  CIPAC MT 75.3 | 5.2 | **5.1** | | Persistent foaming (mL)  After 1 min at 10 % w/v  CIPAC MT 47.2 | 0 | **0** | | Suspensibility (%)  CIPAC MT 184  At 1 %  At 10 % | 60  **98** | **48.5**  **95** | | Dispersibility (%) at 1 % (w/v)  CIPAC MT 174 | **69.5** | **57** | | Wet sieve test (%)  on 75 µm sieve  CIPAC MT 185 | **3.3** | **9.6** | | Wettabillity (s)  CIPAC MT 53.3 | 17.5 | 6.5 | | Dust content (optical factor)  CIPAC MT 171 | 6.42  Nearly dust free | 3.89  Nearly dust free | | Attrition and resistance (%) CIPAC MT 178.2 | **73.79** | **84.05** | | **Microbial contaminants** | | | | Aerobic bacteria  (CFU/g) | < 105 | < 105 | | Yeast and mould  (CFU/g) | <1000 | <1000 | | *Salmonella*  *(Method: study Mo5041)* | Absent in 25 g | Absent in 25 g | | *Shigella*  *(Method: study Mo5041)* | Absent in 25 g | Absent in 25 g | | *E.coli*  *(Method: study Mo5041)* | Absent in 1g | Absent in 1 g | | *Staphylococcus aureus*  *(Method: study Mo5041)* | Absent in 1 g | Absent in 1 g | | *Pseudomonas aeruginosa*  *(Method: study Mo5041)* | Absent in 1g | Absent in 1g |   Aquabac DF 3000 is sensitive to high temperatures. A temperature of 54°C, will kills more than 80 percent of the insecticidal activity of the protein crystal within four hours. This is why the product should be stored temperatures less than 30°C. A 2 year storage study at ambient temperature is in process. | Y | Brux, A. (2015) | Appearance, pH,  persistent foaming  wettability and dust content before and after 14 days at 54 °C can be considered similar to the initial characteristics.  **The suspensibility and the dispersibility are outside the acceptable limits the diluted formulation should be applied under continuous agitation.**    The results of the attrition and resistance of the granules and the wet sieve test are outside the acceptable limits.  **An evidence must be submitted showing the**  **preparation may be satisfactorily applied through appropriate application equipment with no blockages in post registration.**  The microbial contaminants before and after 14 days at 54 °C are in accordance with the OECD 65.  As there is a high difference of biopotency before and after 14 days at 54°C, the Applicant started a new stability test at ambient temperature, see complementary data below  **As no study was provided to prove the stability of the product after exposition to the light, the biocidal product should be protected from the light.** |
|  | Complementary data March 2017 | Aquabac DF 3000  Batch : 1240463 | Packaging: Commercial packaging   |  |  |  | | --- | --- | --- | |  | **T0** | **After 2 years at 20 ± 2 °C** | | LC 50 (µg/mL)/ biopotency (ITU/mg)/ Mortality (%)  (Method: Biopotency (WHO/CDS/WHOPES/GCDPP/2005.12)  (not GLP) | 0.0570  (3000 ITU/mg, 100% mortality) | 0.1328  (2910 ITU/mg,  97% mortality | | Appearance  SOP-PR-015 visually | Solid brown medium grain | Solid brown medium grain | | Stability of the packaging | Test item in sound condition, sealed and without leakages | Test item in sound condition, sealed and without leakages | | pH at ambient temperature ( at 1 %)  CIPAC MT 75.3 | 5.2 | 5.1 | | Persistent foaming (mL)  After 10 s, 1 min, 3 min and 12min at 10 % w/v  CIPAC MT 47.2 | 0 | 0 | | Suspensibility (%)  CIPAC MT 184  At 1 %  At 10 % | 60  98 | 68  100 | | Dispersibility (%) at 1 % (w/v)  CIPAC MT 174 | 69.5 | 70 | | Wet sieve test (%)  on 75 µm sieve  CIPAC MT 185 | **3.3\*** | **5.4\*** | | Wettabillity (s)  CIPAC MT 53.3 | 17.5 | 13 | | Dust content (optical factor)  CIPAC MT 171 | 6.42  Nearly dust free | 6.68  Nearly dust free | | Attrition and resistance (%) CIPAC MT 178.2 | **73.79\*** | **93.00\*** | | **Microbial contaminants** | | | | Aerobic bacteria  (CFU/g)  *(Method: study Mo5041)* | <105 | 1.70 × 104 | | Yeast and mould  (CFU/g)  *(Method: study Mo5041)* | <10 | <10 | | *Salmonella*  *(Method: study Mo5041)* | Absent in 25 g | Absent in 25 g | | *Shigella*  *(Method: study Mo5041)* | Absent in 25 g | Absent in 25 g | | *E.coli*  *(Method: study Mo5041)* | Absent in 1g | Absent in 1 g | | *Staphylococcus aureus*  *(Method: study Mo5041)* | Absent in 1 g | Absent in 1 g | | *Pseudomonas aeruginosa*  *(Method: study Mo5041)* | Absent in 1g | Absent in 1g |   \*According to the Applicant:  - the results of the wet sieve test were above 2% before and after storage. However, in the field there have been no reported problems with the current formulation (according to study monitor).  Note: Aquabac DF3000 does not dissolve. It forms a suspension and is insoluble in water.  - The results of the attrition resistance were below 98 % before and after storage. There were problems with the test causing the granules to breakup during the testing, thereby causing blockages in the sieve. Multiple sieving was attempted (non-GLP), but this only lead to the granules breaking up further. Therefore, the results are not representative of the product. | Y | Manka S., 2017 | Acceptable  Appearance, pH,  persistent foaming, the suspensibility, the dispersibility  wettability and dust content before and after 2 years at 20 ±2 °C can be considered similar to the initial characteristics.  The results of the attrition and resistance of the granules and the wet sieve test are outside the acceptable limits. **An evidence must be submitted showing the**  **product may be satisfactorily applied through appropriate application equipment with no blockage in post registration.**  For the attrition and resistance considering the mitigation, measures (mask) more data required.  The microbial contaminants before and after 2 years at 20 °C are in accordance with the OECD 65.  The biopotency decrease slightly after 2 years storage at 20 °C and is below the minimum certified value, nevertheless, as the efficacy is near to T0 (97 % mortality), no more data required. |
| **IIIB 2.2.2 Other factors affecting stability (B. 3.5)** | No other information regarding stability is required or has been submitted. | | | | |  |
| **IIIB 2.3 Explosivity (B.4.1)and oxidising properties (B.4.9)** | Theoretical assessment | Not relevant | None of the components of this formulation are classified as having explosive properties. Therefore this study has not been performed | / | / | Acceptable |
| Theoretical assessment | Not relevant | None of the components of this formulation are classified as oxidising. Therefore this study has not been performed. | / | / | Acceptable |
| **IIIB 2.4 Flashpoint**  **and other indications of flammability(B.4.7) or spontaneous ignition (B.4.7)** | Theoretical assessment | Not relevant | AQUABAC DF 3000 is not a liquid formulation and therefore flash point is not relevant.  Flammability was not performed as none of the components are classified as flammable.  Self ignition: no information provided |  |  | Acceptable  No information was provided on the self ignition of the biocidal product Aquabac DF 3000, nevertheless, considering the composition of the biocidal product it is not expected to be auto-flammable at ambient temperature. |
| **IIIB 2.5 Acidity, alkalinity, pH value, pour and bulk density** | CIPAC MT75.3  CIPAC MT 159 and OECD TG 09 | Aquabac DF 3000  Batch: 1240463  a.i. content not specified | pH = 4.97 at 1 % dilution and at ambient temperature.  Pour density: 0.554 g/mL  Tap density: 0.644 g/mL | Y | Wo (2013) | Acceptable |
| **IIIB 2.6 Viscosity and surface tension** | Aquabac DF 3000 is not a liquid formulation and therefore viscosity and surface tension are not relevant. | | | | | Acceptable |
| **IIIB 2.7** | **Technical characteristics** | | | | |  |
| **IIIB 2.7.1 Wettability** | House method equivalent to CIPAC MT 53.3 | Aquabac DF3000  Batches :  0960433  100453  1240463  1250463 | 1-5 seconds at 20°C | N | Becker Microbial (2013) | Acceptable |
|  | Complementary data March 2015  CIPAC MT 53.3 | Aquabac DF 3000  Batch : 2141814 | 17.5 s | Y | Brux, A. (2015) | Acceptable |
| **IIIB 2.7.2 Persistent foaming** | / | / | Not performed as the product is a granule | / | / | The persistent foaming at the maximum use concentration was provided in complementary data. See below. |
| Complementary data March 2015  CIPAC MT 47.2 | Aquabac DF 3000  Batch : 2141814  CIPAC MT 46.3 | At 10 % v/v dilution   |  |  | | --- | --- | | Time | Foam (mL) | | After 10 sec | 28 | | After1 min | 0 | | After 3 min | 0 | | After 12 min | 0 | | Y | Brux, A. (2015) | Acceptable  The persistent foaming is in the acceptable limits at 10 % (v/v). |
| **IIIB 2.7.3 Suspensibility , suspension stability and dispersibility** | Suspensibility House method  Dispersibility | / | Suspensibility at 5 % (w/v): good dispersibility  Dispersibility: Not performed as the product is a granule | / | / | A new suspensibility test with the CIPAC MT 184 at the minimum and at the maximum use concentration was provided in complementary data. See below.  A new dispersibility test with CIPAC MT 174 at the maximum use concentration is provided in complementary data. See below. |
| Suspensibility  Complementary data March 2015 | Aquabac DF 3000  Batch : 2141814  CIPAC MT 184 | At 1 %: 60 %  At 10 %: 98 % | Y | Brux, A. (2015) | Acceptable |
| Dispersibility  Complementary data March 2015 | Aquabac DF 3000  Batch : 2141814  CIPAC MT 174 | At 1 %: 69.5. % | Y | Brux, A. (2015) | Acceptable at 1 %  The test at the maximum use concentration is missing , nevertheless, as the application should be performed continuous agitation, no more data required. |
| **IIIB 2.7.4 Wet sieve and dry sieve test** | In-house method equivalent to CIPAC MT 185 | Aquabac DF3000  Batches  0960433  1000433  1240453  1250463 | Average 0.5% residue through 75µm wet sieve.  Average 54.9% residue through a 150 µm dry sieve.  Average 0.2% residue through a 1180 µm dry sieve | / | Becker Microbial (2013) | Acceptable |
| Complementary data March 2015  CIPAC MT 185 | Aquabac DF 3000  Batch : 2141814 | On 75 µm sieve: 3.3 % | Y | Brux, A. (2015) | Not acceptable  The wet sieve test is outside the acceptable limits. A statement was provided below but was considered as not acceptable. **An evidence must be submitted showing that the product may be satisfactorily applied through appropriate application equipment with no blockages in post registration.** |
| Statement and efficacy test (EID 14087 report)  Complementary data June 2019 |  | A Statement and an efficacy test (EID 14087 report) were provided to demonstrate that no blockage of the application equipement is posssible:  The Notifier indicate that the mixture is maintained under agitaion by shaking of the application equipment during treatment. Furthermore, he indicates that the the maximum dilution is 10 % and in this case the the blockage of the material is not observed and results are in accordance with results of the efficacy test study EID 14087. |  |  | This information is not considered sufficient to demonstrate that there is no blockage of the application equipment. |
| **IIIB 2.7.5 Particle size distribution, content of dust/fines, attrition and friability** | Particle size distribution | / | Not performed as the product is a wettable granular | / | / | Acceptable |
| Dustiness  House method | Aquabac DF3000  Batches  0960433  1000433  1240453  1250463 | 150 µm to 1180 µm | Y | Becker Microbial (2013) | Not acceptable,  The method was not provided; A study of dustiness using CIPAC MT 171 was required and is provided in complementary data. See below. |
| Dust content  Complementary data March 2015  CIPAC MT 171 | Aquabac DF 3000  Batch : 2141814 | Optical factor: 6.42  The product is nearly dust free. | Y | Brux, A. (2015) | Acceptable |
| Attrition and resistance  Complementary data March 2015  CIPAC MT 178.2 | Aquabac DF 3000  Batch : 2141814 | 73.79 % | Y | Brux, A. (2015) | The results of the attrition and resistance of the granules is outside the acceptable limits **an evidence must be submitted showing the**  **product may be satisfactorily applied through appropriate application equipment.** |
| **IIIB 2.7.6 Emulsifiability, re-emulsifiability, emulsion stability** | Aquabac DF 3000 is not an emulsifiable formulation and therefore emulsion characteristics are not relevant. | | | | | Acceptable |
| **IIIB 2.7.7 Flowability, pourability and dustability** | / | / |  |  |  | Not required for wettable granular. |
| **IIIB 2.8** | **Physical, chemical and biological compatibility with other products** | | | | |  |
| **IIIB 2.8.1 Physical compatibility** | Aquabac DF 3000 is not intended for application as a tank mixed formulation and therefore information regarding compatibility with other production is not relevant. | | | | | Acceptable |
| **IIIB 2.8.2 Chemical compatibility** | Aquabac DF 3000 is not intended for application as a tank mixed formulation and therefore information regarding compatibility with other production is not relevant. | | | | | Acceptable |
| **IIIB 2.8.3 Biological compatibility** | Aquabac DF 3000 is not intended for application as a tank mixed formulation and therefore information regarding compatibility with other production is not relevant. | | | | | Acceptable |
| **IIIB 2.9 Summary and evaluation of physical, chemical and technical properties** | The biocidal product ”AQUABAC DF 3000” is Wettable Granules (WG). The appearance of the formulation is light tan granules with fresh acidic fermentation odour. It is not explosive and has no oxidizing properties. It is not flammable and not auto-flammable at ambient temperature. In aqueous solution (1% dilution), its pH is 5 at ambient temperature.  The persistent foaming was found in the acceptable limits at 10 % (w/v).  The suspensibility and the dispersibility are outside the acceptable limits, so, **the diluted formulation should be applied under continuous agitation.**  The wettability of the granule is in the acceptable limits.  The results of the attrition and resistance of the granules and the wet sieve test are outside the acceptable limits. **An evidence must be submitted showing the product may be satisfactorily applied through appropriate application equipment with no blockages in post registration. These data will be required at the renewal of the authorisation.**  The storage at 54 °C for 14 days indicates that the microbial active substance is not stable at this temperature. **A 2-year stability study indicactes that biocidal product AQUABAC DF 3000 in its commercial packaging (HDPE) is stable for 2 years at ambient temperature in its commercial packaging. Compatibility with carton box is acceptable by extrapolation.**  **The product should not be stored at temperature higher than 20°C.**  **In absence of information on the stability after exposition to the light, the biocidal product should be protected from the light.**  The biocidal product AQUABAC DF 3000 is not classified for the physical-chemical part. | | | | |  |

* + - 1. **Analytical method for determining the active substance and relevant component in the biocidal product**

**2.3.2.3.1 Methods for microbial active substance**

Reference: Becker Microbial Products (2005) BMP 144 Purity Plating, Unpublished report, Sponsor: Becker Microbial Products, Inc., protected, Not GLP.

**Principle of the method**

Petri dishes containing pre-poured Brain Heart Infusion medium were inoculated with seed stock samples before inoculation using a sterile loop. The loops were used to streak the plate in a specific pattern.

Contaminates and phage in the confluent growth area were assessed after 12, 48 and 78 hours incubation at 30°C and 37°C. At least two plates per sample was measured. If contamination is present it can be observed in the streak lines.

**Results**

No validation data were provided.

**Conclusion**

The method provided was not validated.

Reference: Benzon Research (2001) Potency Bioassay for *Bacillus thuringiensis* *israelensis*, unpublished report, Sponsor: Becker Microbial Products, Inc., data protection: yes, GLP

**Principle of the method**

Laboratory strains of *Aedes aegypti* (L.) with no known resistance to insecticides are cultured for the study. Larvae of late 3rd or early 4th instar, less than 24 hours old are used in the study. At least 5 concentrations for each sample shall be made in non-chlorinated or dechlorinated tap water. Three cups each containing 20 larvae and an un-dosed control cup were evaluated for 24 hours (27±2°C & Light:Dark 16:10 hrs).

The number of dead larvae exposed to dose concentrations of *Bti* are counted after 24 hours and are compared to a reference standard of *Bti* H-14 calibrated against the International Standard IPS-82 (11,000 ITU/mg). Statistical analysis to determine the potency of each sample was conducted by Probit analysis.

Potency of sample in ITU/mg = LC50 ref standard X Potency of ref standard in ITU/mg

LC50 of sample

**Results**

**Repeatability:** No validation data were provided

**Conclusion**

The method provided was not validated.

Reference: Brux, A, 2015, Determination of physico-chemical properties and storage stability tests for Aquabac DF 3000 Study: Mo5041, Biogenus

**Principle**

The test is performed in the laboratory at ambient temperature (24°C) and relative humidity (65%), photo period approx.. 10 hours : 14 hours.

To achive the adequate concentrations for each product, a stock solution is made which is diluted with tap water down to desired concentrations.

90 ml of each solution is filled into plastic cups which have been labelled before.

Untreated cups with tap water serve as control.

20 mosquito larvae (stage 3) are introduced into each cup. The cups are covered with netting to prevent the emergence and escape of adults into environment. The percentage of dead larvae is recorded after 24 hours. No water was replenished. No food was provided. Each test consisted of 3 replicates of which the mean values are calculated.

**Results**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Mortality of larvae**(*A. aegypti*) **after (%) with the following dosages at T0** | | | | | | | LC 50 |
| **Rep** | **0.3124  mcg/ml** | **0.2187  mcg/ml** | **0.1531  mcg/ml** | **0.1071  mcg/ml** | **0.075  mcg/ml** | **0.0525  mcg/ml** | **0.0368  mcg/ml** |  |
| 1 | 100 | 95 | 90 | 75 | 60 | 40 | 30 | 0.0570 mcg/ml |
| 2 | 100 | 100 | 95 | 80 | 75 | 70 | 20 |
| 3 | 100 | 100 | 100 | 90 | 50 | 40 | 20 |
| Mean | 100 | 98 | 95 | 82 | 62 | 50 | 23 |
| SD | 0.0 | 2.9 | 5.0 | 7.6 | 12.6 | 17.3 | 5.8 |
| %RSD | 0.0 | 2.9 | 5.3 | 9.4 | 20.4 | 34.6 | 24.7 |
|  |  |  |  |  |  |  |  |  |
|  | **Mortality of larvae** (*A. aegypti*) **after (%) with the following dosages at 54 °C  after 2 weeks storage** | | | | | | | LC 50 |
| **Rep** | **0.3111  mcg/ml** | **0.2187  mcg/ml** | **0.1531  mcg/ml** | **0.1071  mcg/ml** | **0.075  mcg/ml** | **0.0525  mcg/ml** | **0.0368  mcg/ml** |  |
| 1 | 100 | 90 | 90 | 70 | 55 | 30 | 20 | 0.0716 mcg/ml |
| 2 | 100 | 100 | 90 | 70 | 60 | 20 | 10 |
| 3 | 100 | 90 | 90 | 70 | 55 | 30 | 20 |
| Mean | 100 | 93 | 90 | 70 | 57 | 27 | 17 |
| SD | 0.0 | 5.8 | 0.0 | 0.0 | 2.9 | 5.8 | 5.8 |
| %RSD | 0.0 | 6.2 | 0.0 | 0.0 | 5.1 | 21.7 | 34.6 |

|  |  |  |
| --- | --- | --- |
|  | % knock down/mortakity of larvae (*A. aegypti*) | |
| **Rep** | **Control 1** | **Control 2** |
| 1 | 0 | 0 |
| 2 | 0 | 0 |
| 3 | 0 | 0 |
| Mean | 0 | 0 |

**Conclusion**

Validation data obtained (%RSD and controls) can be considered sufficient.

**Nevertheless, the determination of the microbial active substance in five batches of the biocidal product AQUABAC DF 3000 is missing and is required in post registration. These data will be required at the renewal of the authorisation.**

**2.3.2.3.1 Methods for microbial contaminants**

Reference: Brux, A, 2015, Determination of physico-chemical properties and storage stability tests for Aquabac DF 3000 Study: Mo5041, Biogenus

Detail of the determination of microbial contaminants in one batch of the biocidal product AQUABAC DF3000 (batch: 1240463) is summarised in the table below.

**Table of detailed contaminants in 1 representative batch of AQUABAC DF 3000**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Microbial contaminants at T=0 | | Microbial contaminants after 14 days  at 54°C | | Certified  values |
|  | Replicate 1 | Replicate 2 | Replicate 1 | Replicate2 |  |
| *Aerobic  bacterial contaminants* cfu/g | <1E+5 | <1E+6 | <1E+7 | <1E+8 | <1E+5 |
| *Yeast and mould* | <10 | <11 | <12 | <13 | <1E+3 |
| *Salmonella* | Absent in 25g | Absent in 25g | Absent in 25g | Absent in 25g | Absent in 25g |
| *Shigella* | None  recovered in 25 g | None  recovered in 25 g | None  recovered in 25 g | None  recovered in 25 g | Absent in 25g |
| *E.Coli* | Absent in 1g | Absent in 1g | Absent in 1g | Absent in 1g | Absent in 1g |
| *Staphylococcus  aureus* | Absent in 1g | Absent in 1g | Absent in 1g | Absent in 1g | Absent in 1g |
| *Psedomonas  aeroginosa* | Absent in 1g | Absent in 1g | Absent in 1g | Absent in 1g | Absent in 1g |

Methods used for the determination of each microbial contaminant in the above table are summarised below.

1. **Quantitative determination of aerobic bacterial contaminants**

1 g (±0.01 g) of the test substance was aseptically transferred into 99 mL BSCPT. This BSCPT + test substance suspension (= Group A) was then inoculated with 0.1 mL (containing 5 x 104 viable cells) of *Bacillus cereus* inoculum. Duplicate containers of 99 mL BSCPT with 1 g test substance without any inoculated validation organism (= Group C) was prepared to determine the presence of aerobic bacterial contaminants in the test substance. This 'Group C' preparation was further diluted in ten-fold steps using BSCPT down to 10-8. An additional container of 99 mL BSCPT without test substance (= Group B) was similarly inoculated as a diluent inoculum control. A portion (0.1 mL) of the 1:99 w/v test substance suspensions and further dilutions to 10-8 were spread in triplicate over the surface of TSA plates. The TSA plates were incubated at 30 — 35°C for to 3 to 5 days and then the numbers of Bacillus cereus colonies present on the plates was recorded.

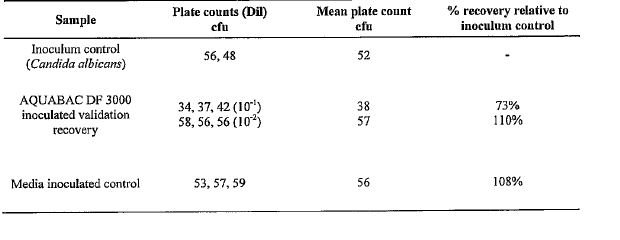
**Validation data**



**2. Quantitative determination of Total Yeasts and Moulds Count (TYMC)**

1 g of the test substance was added to 9 mL BSCPT to give a IO"' dilution. A further ten-fold dilution was prepared to give a 10-2 dilution. These BSCPT + test material broths were then each inoculated with 0.1 mL (containing 100 — 1000 viable cells) of Candida albicans inoculum. A second replicate was prepared in this manner and inoculated with 0.1 mL (containing 100 —1000 viable spores) of Aspergillus braziliensis inoculum. Duplicate containers of 9 mL BSCPT with 1 g test substance without any inoculated validation organisms were prepared to determine the presence of contaminating yeast and mould organisms in the test substance. Additional containers of 9 mL BSCPT without test substance were separately inoculated with Candida albicans and Aspergillus braziliensis as a diluent control group. Triplicate 1 mL aliquots from each dilution were used to prepare agar pour plates on SDA containing 100 p.g/mLchloramphenicol. The SDA plates were incubated for up to 5 days at 20 — 25°C and then die numbers of yeast and mould colonies present on die plates were recorded.

**Validation data**



****

**3. Qualitative determination of Salmonella**

25 g of the test substance was added to 225 mL Lactose Broth (LB, Oxoid CM0137) to give a 104 dilution. This LB + test substance broth was then inoculated with 0.1 mL (containing 10 —100 viable cells) of Salmonella abony inoculum. Duplicate containers of 225 mL LB broth with 25 g test substance without any inoculated validation organism was prepared to determine the presence of contaminating Salmonella in the test substance. An additional container of 225 mL LB without test substance was similarly inoculated as a Sahnonella media inoculum control. The LB broths were incubated for 18 - 24 hours at 30 — 35°C. One mL aliquots of the incubated LB broths were then transferred into 10 mL Rappaport Vassiliadis Broth (RV, Oxoid CM0669) and incubated for 18 - 24 hours at 30 — 35°C. Each incubated RV broth was then streaked onto Xylose Lysine Deoxycholate Agar (XLD, Oxoid CM0469) in triplicate and incubated for 18 - 48 hours at 30 — 35°C and then the plates examined for the presence of Salmonella colonies.

**Validation data**



**4. Qualitative determination of *Shigella***

25 g of the test substance was added to 225 mL LB to give a 10-1 dilution. This LB + test substance broth was then inoculated with 0.1 mL (containing 10 —100 viable cells) of Shigella sonnei inoculum. The duplicate containers of 225 mL LB broth with 25 g test substance without any inoculated validation organism (prepared in 4.3.3 above) was used to determine the presence of contaminating Shigella in the test substance. An additional container of 225 mL LB without test substance was similarly inoculated as a Shigella media inoculum control. The LB broths were incubated for 18 - 24 hours at 30 — 35°C. The incubated LB broths were then streaked onto Xylose Lysine Deoxycholate Agar (XLD, Oxoid CM0469) in triplicate and incubated for 18 - 48 hours at 30 — 35°C and then the plates examined for the presence of Shigella colonies.

**Validation data**



**5. Qualitative determination of *E. coli***

1 g of the test substance was added to 9 mL LB to give a 10-1 dilution. This LB + test substance broth was then inoculated with 0.1 mL (containing 10 —100 viable cells) of E. coli inoculum. Duplicate containers of 9 mL LB broth with 1 g test substance without any inoculated validation organism were prepared to determine the presence of contaminating E. coli in the test substance. An additional container of 9 mL LB without test substance was similarly inoculated as an E. coli media inoculum control. The LB broths were incubated for 18 - 24 hours at 30 — 35°C. 1 mL from the incubated LB broths were transferred into 100 mL MacConkey broth (MacB, Oxoid CM0005) and incubated at 42 — 44°C for 24 - 48 hours. The incubated MacB broths were then streaked onto MacConkey Agar (Mac, Oxoid CM0007) plates and incubated for 18 - 72 hours at 30 — 35°C and then the plates examined for the presence of E. coli colonies.

**Validation data**



**6. Qualitative determination of *Staphylococcus aureus***

1 g of the test substance was added to 100 mL Tryptone Soya Broth (TSB, Oxoid CM0129). This TSB + test substance broth was then inoculated with 0.1 mL (containing 10 —100 viable cells) of Staphylococcus aureus inoculum. Duplicate containers of 100 mL TSB broth with 1 g test substance without any inoculated validation organism were prepared to determine the presence of contaminating Staphylococcus in the test substance. An additional container of 100 mL TSB without test substance was similarly inoculated as a Staphylococcus media inoculum control. The TSB broths were incubated for 18 - 24 hours at 30 — 35°C. The incubated TSB broths were then streaked onto a Mannitol Salt Agar plate (MSA - Oxoid CM0085) and incubated at 30 - 35°C for at least 18 - 72 hours and then the plates examined for the presence of typical Staphylococcus aureus colonies.

**Validation data**



**5. Qualitative determination of *Pseudomonas aeruginosa***

1 g of the test substance was added to 100 mL TSB. This TSB + test substance broth was then inoculated with 0.1 mL (containing 10 — 100 viable tells) of Pseudomonas aeruginosa inoculum. Duplicate containers of 100 mL TSB broth with 1 g test substance without any inoculated validation organism were prepared to determine the presence of contaminating Pseudomonas aeruginosa in the test substance. An additional container of 100 mL TSB without test substance was similarly inoculated as a Pseudomonas aeruginosa media inoculum control. The TSB broths were incubated for 18 - 24 hours at 30 — 35°C. The incubated TSB broths were then streaked onto Cetrimide Agar (CET, Oxoid CM0579) and incubated at 30 —35°C for at 18 — 72 hours and then the plates examined for the presence of typical.

**Validation data**



Waivers were provided by the Notifier for the other microbial contaminants indicated in OECD 65 (Oct. 2011) and are summarized below:

1) *Listeria monocytogenes*

As the routine analysis of *Bti* demonstrates that other hygiene indicators are at a very low level and furthermore the entire process manufacture of *Bti* takes place in a sterile system to guarantee quality and purity of production, the waiver of this test is in accordance with OECD 65.

2) *Vibrio*

As the entire process manufacture of *Bti* takes place in a sterile system to guarantee quality and purity of production, and the manufacture does not occur in a geographical location where *Vibrio* is naturally occurring, the waiver of this test is in accordance with OECD 65.

3) Anaerobic spore-formers

As the routine analysis of *Bti* demonstrates that both *Escherichia coli* and *Staphylococcus aureus* are analysed and present at a very low level and furthermore the entire process manufacture of *Bti* takes place in a sterile system to guarantee quality and purity of production, the waiver of this test is in accordance with OECD 65.

**Conclusion**:

Certified values for each microbial contaminant set by the Notifier are in accordance with OECD 65 (Oct. 2011).

Validation data provided for each microbial contaminant indicated in OECD 65 (Oct. 2011) are considered sufficient.

**The determination of microbial contaminants was performed only in one batch of the biocidal product AQUABAC DF 3000, a new determination in 5 batches was provided and is summarised below.**

Reference: Anonymous, 2014, Review of microbial contamination methods for Bti (BMP 144), JSC International Limited0, Sponsor: Cera.

Reference: Anonymous, 2014, Validation results for Microbial Contamination methods conducted on Bti (BMP 144), JSC International, Non-GLP Unpublished, Sponsor: Cera.

The Notifier indicates the certified values and the international standard methods used for the determination of microbial contaminants in its biocidal products. This information is summarised below:

|  |  |  |  |
| --- | --- | --- | --- |
|  | Certified value | Threshold level according to OECD Issue paper 65 | Method used |
| **Coliform in 1 g (CFU/g)** | <10 CFU/g | < 10 CFU/g | AOAC 991.14 & 998.08 |
| **E. Coli in 1 g (CFU/g)** | **<10 CFU/g** | **Absence in 1 g** | AOAC 991.14 & 998.08 |
| **Enterococcus KF  (CFU/g)** | < 10 CFU/g | - | CMMEF Chapter 9 |
| **Pseudomonas  Aeroginosa in 1 g and more** | Absent | Monitoring | USP 35 Method 62 |
| **Salmonella per 25g** | Absent | Absence in 25 g | BAM Chapter 5 |
| **Shigella, per 25g** | Absent | Absence in 25 g | CMMEF Chapter 38 |
| **Staphylococcus  aureus, per 25g (USP)** | Absent | Absence in 1 g | USP 35 Method 62 |
| **Mould (CFU/g)** | <10 CFU/g | < 1000 CFU/g | AOAC 997.02 |
| **Yeast  (CFU/g)** | <10 CFU/g | < 1000 CFU/g | AOAC 997.02 |

**Conclusion**:

Certified values set by the Notifier are in accordance with OECD 65 (Oct. 2011) except for *E.Coli*, nevertheless, as the certified values for coliform is in accordance with OECD 65 (Oct. 2011), no more data required.

Methods provided for the determination of microbial contaminants are international standard methods.

No more data required.

**The microbial contaminants were determined only on one batch, the determination of microbial contaminants in five batches of the biocidal product AQUABAC DF 3000 according to OECD 65 was provided and is summarised below.**

Reference: Akhurst L.C., 2016, AQUABAC DF3000: 5 batch analysis for microbial contaminants, CFU count and specificity, Report: NR49CV, Sponsor: CERA SAS, GLP

**Methods**

The methods provided for the determination of the microbial active substance and for the determination of microbial contaminants are similar with those provided for the authorisation of the product with minor modifications. These methods were already validated and are reported below.

CFU count:

1 g (±0.01 g) of each batch of test item was aseptically transferred into separate 99 mL volumes of Buffered Sodium Chloride Peptone solution containing 0.1% Tween 80 (BSCPT, Oxoid CM0982) and homogenised for 30 seconds using an Ultra Turrax blender. A portion (0.1 mL) of the 1:99 w/v test item suspension and further dilutions to 10-5 were spread in triplicate over the surface of Tryptone Soya Agar (TSA, Oxoid CM0131) plates for determination of aerobic bacterial contaminants. In addition, 5 replicates of 0.1 mL from further dilutions to 10-8 were spread over the surface of TSA plates for viable cfu count of the test item. The TSA plates were incubated at 30 — 35°C for 2 days and then the numbers test item colonies present on the plates were recorded for the `cfu' count and the numbers of aerobic bacterial contaminant colonies present recorded.

Yeasts and Moulds Count

1 g of each batch of test item was added to separate 9 mL BSCPT to give a 10-1 dilution. A further ten-fold dilution was prepared to give a 10-2 dilution. Triplicate 1 mL aliquots from each dilution were used to prepare agar pour plates on Sabouraud Dextrose Agar (SDA, Oxoid CM0041) containing 100 µg/mL chloramphenicol. The SDA plates were incubated for 3 days at 20 — 25°C and then the numbers of yeast and mould colonies present on the plates were recorded.

*Salmonella*

25 g of each batch of test item was added to separate 225 mL Lactose Broth (LB, Oxoid CM0137) to give a 10-1 dilution. The LB broths were incubated for 18 - 24 hours at 30 — 35°C. A 1 mL aliquot of each incubated LB broth was then transferred into 10 mL Rappaport Vassiliadis Broth (RV, Oxoid CM0669) and incubated for 18 - 24 hours at 30 — 35°C. The incubated RV broths were then streaked onto Xylose Lysine Deoxycholate Agar (XLD, Oxoid CM0469) and incubated for 18 - 48 hours at 30 — 35°C and then the plates examined for the presence of *Salmonella* colonies.

*Shigella*

25 g of each batch of test item was added to separate 225 mL LB (the LB broths prepared in the above paragraph). The LB broths were incubated for 18 - 24 hours at 30 — 35°C. The incubated LB broths were then streaked onto Xylose Lysine Deoxycholate Agar (XLD, Oxoid CM0469) and incubated for 18 - 48 hours at 30 — 35°C and then the plates examined for the presence of *Shigella* colonies.

*E. coli*

1 g of each batch of test item was added to separate 9 mL LB. The LB broths were incubated for 18 - 24 hours at 30 — 35°C. A 1 mL aliquot from the incubated LB broths was then transferred into 100 mL MacConkey broth (MacB, Oxoid CM0005) and incubated at 42 — 44°C for 24 - 48 hours. The incubated MacB broths were then streaked onto MacConkey Agar (Mac, Oxoid CM0007) and incubated for 18 - 72 hours at 30 — 35°C and then the MacConkey Agar examined for the presence of *E. coli* colonies.

Staphylococcus aureus

1 g of each batch of test item was added to separate 100 mL Tryptone Soya Broth (TSB, Oxoid CM0129). The TSB broths were incubated for 18 - 24 hours at 30 — 35°C. The incubated TSB broths were then streaked onto a Mannitol Salt Agar plate (MSA - Oxoid CM0085) and incubated at 30-35°C for 18 - 72 hours and then the plate examined for the presence of typical *Staphylococcus aureus* colonies.

Pseudomonas aeruginosa

1 g of each batch of test item was added to separate 100 mL TSB (the TSB broths prepared in the above paragraph were used). The TSB broths were incubated for 18 - 24 hours at 30 — 35°C. The incubated TSB broths were then streaked onto Cetrimide Agar (CET, Oxoid CM0579) and incubated at 30 - 35°C for 18 — 72 hours and then the plate examined for the presence of typical *Pseudomonas aeruginosa* colonies.

**Assessment of the nutritive/selective properties of the selective test media**

The following micro-organisms were used to confirm the nutritive/selective properties of the appropriate media used in this study.

|  |  |
| --- | --- |
| *Aspergillus brasiliensis Candida albicans*  *Bacillus cereus*  *Escherichia coli*  *Salmonella abony*  *Shigella sonnei Pseudomonas aeruginosa Staphylococcus aureus* | ATCC16404  ATCC 10231  NCTC 11143  NCIMB 8545 (ATCC 8739)  NCTC 6017  NCTC 8574  NCIMB 8626 (ATCC 9027)  NCIMB 9518 (ATCC 6538) |

**Results**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Batch 0730353** | **Batch**  **0960433** | **Batch 1240463** | **Batch 1250463** | **Batch 2531732** | **Certified values** | **Threshold level according to OECD Issue paper 65 for contaminants** |
| Spores (CFU/g) | 2.02 x 1010 | 3.74 x 1010 | 3.46 x 1010 | 1.63 x 1010 | 1.54 x 1010 | Minimum: 1.54 × 1010 CFU/g  Nominal: 2.48 × 1010 CFU/g  Maximum: 3.74 × 1010 CFU/g | / |
| Individual plate count (dilution counted) | 48, 16, 12, 14, 11 (-8) | 35, 33, 41, 38, 40 (-8) | 29, 28, 45, 47, 24 (-8) | 135, 231, 228, 185, 37 (-7) | 135, 85, 118, 97, 334 (-7) |
| Aerobic bacterial count (CFU/g) | <1000 | <1000 | <1000 | <1000 | <1000 | 105 CFU/g | 105 CFU/g |
| Yeast & mould count (CFU/g) | 4.67X103 | **1.07X104** | 7.33X103 | **2.37X104** | **4.60X104** | < 1000 CFU/g | < 1000 CFU/g |
| *Salmonella* | Absent in 25 g | Absent in 25 g | Absent in 25 g | Absent in 25 g | Absent in 25 g | Absent in 25 g | Absent in 25 g |
| *Shigella* | Absent in 25 g | Absent in 25 g | Absent in 25 g | Absent in 25 g | Absent in 25 g | Absent in 25 g | Absent in 25 g |
| *E. coli* | Absent in 1 g | Absent in 1 g | Absent in 1 g | Absent in 1 g | Absent in 1 g | Absent in 1 g | Absent in 1 g |
| *Staphylococcus aureus* | Absent in 1 g | Absent in 1 g | Absent in 1 g | Absent in 1 g | Absent in 1 g | Absent in 1 g | Absent in 1 g |
| *Pseudomonas aeruginosa* | Absent in 1 g | Absent in 1 g | Absent in 1 g | Absent in 1 g | Absent in 1 g | Monitoring | Monitoring |

Satisfactory growth was observed for the control micro-organisms used to confirm the nutritive/selective properties of the appropriate media employed in this study. The results for the assessment of nutritive/selective properties are shown in the below Table 3.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Test** | **Media tested** | **QC organism** | **Growth or count of QC organism** | | | | |
| **Count** | **Mean** | **TSA count**  **(SDA for yeast and moulds)** | **Mean** | **% recovery** |
| Aerobic bacterial plate count | TSA | 1. *cereus* | N/A | N/A | 83, 78 | 81 | N/A |
| Total yeasts and moulds  count | SDAC | *Asp. brasiliensis* | *71, 71* | 71 | 73, 74 | 74 | 96 |
| 1. *albicans* | 82, 81 | 82 | 89, 67 | 78 | 105 |
| *Salmonella* | LB/RV/XLD | *Salmonella abony* | Growth m media | | 169, 164 | 167 | N/A |
| *Shigella* | LB/XLD | *Shigella sonnei* | Growth in media | | 119, 102 | 111 | N/A |
| *Escherichia coli* | LB/MacB/MacA | *E. coli* | Growth in media | | 285, 279 | 282 | N/A |
| *Staphylococcus aureus* | TSB/MSA | *S. aureus* | Growth in media | | 95, 76 | 86 | N/A |
| *Pseudomonas aeruginosa* | TSB/CET | *P. aeruginosa* | Growth in media | | 131, 135 | 133 | N/A |

**Conclusions:**

The content of the microbial active substance in five batches of the product AQUABAC 300DF is in accordance with the minimum certified value in terms of ITU/g. No determination was performed in term of biopotency.

The content of the microbial contaminants in the five batches of the product AQUABAC DF 3000 is in accordance with the certified values and with the document OECD 65 except for yeast and mould in three batches. **As the yeast and mould are outside the acceptable limits in three batches, batches which are not in accordance with the limits indicated in the document OECD 65 should not be commercialised.**

#### Analytical methods for determining relevant components and/or residues in different matrices

Analytical methods for the determination of residue in plants, food of animal origin, body fluids, soil, water and air are not considered necessary, as no residue definition and no MRL were set.

## Risk assessment for physico-chemical properties and analytical methods

The biocidal product ”AQUABAC DF 3000” is Wettable Granules (WG). The appearance of the formulation is light tan granules with fresh acidic fermentation odour. It is not explosive and has no oxidizing properties. It is not flammable and not auto-flammable at ambient temperature. In aqueous solution (1% dilution), its pH is 5 at ambient temperature.

The persistent foaming was found in the acceptable limits at 10 % (w/v).

The suspensibility and the dispersibility are outside the acceptable limits, so, **the diluted formulation should be applied under continuous agitation.**

The wettability of the granule is in the acceptable limits.

The results of the attrition and resistance of the granules and the wet sieve test are outside the acceptable limits. **An evidence must be submitted showing the product may be satisfactorily applied through appropriate application equipment with no blockages in post registration. These data will be required at the renewal of the authorisation.**

The storage at 54 °C for 14 days indicates that the microbial active substance is not stable at this temperature. **A 2-year stability study indicactes that biocidal product AQUABAC DF 3000 in its commercial packaging (HDPE) is stable for 2 years at ambient temperature in its commercial packaging.**

**The product should not be stored at temperature higher than 20°C.**

**In absence of information on the stability after exposition to the light, the biocidal product should be protected from the light.**

The biocidal product AQUABAC DF 3000 is not classified for the physical-chemical part.

Analytical methods for the determination of the microbial active substance and microbial contaminants in the product AQUABAC 200 G were provided and were considered validated.

The determination of the biopotency in five batches of the product AQUABAC DF 3000 using validated method was not performed and should be provided in post registration. **These data will be required at the renewal of the authorisation.**

***Measures*** *linked to assessment of physico-chemical properties*

|  |
| --- |
| Shelf life: 24 months  Do not store above 20° C.  ***Required information linked to*** *physico-chemical properties and analytical methods* ***at the renewal of the authorisation*** |

* An evidence must be submitted showing the product may be satisfactorily applied through appropriate application equipment with no blockages.
* The determination of the biopotency in five batches of the product AQUABAC DF 3000 using validated method was not performed and should be provided.

## Effectiveness against target organisms

### Function

MG 03: Pest Control

Product Type 18: Insecticides, acaricides and products to control other arthropods.

AQUABAC DF 3000 is presented as water dispersible granules (WG). The formulation has a potency of 3000 IUT/mg and contains 43 % w/w of the insecticidal micro-organism Bti strain BMP 144.

The biocidal product AQUABAC DF3000 is a larvicide used by professional operators, for the control of mosquito larvae in water habitats. The product is applied by air or through ground application.

### Organisms to be controlled and products, organisms or objects to be protected

According to the uses claimed by the applicant, AQUABAC DF3000 is intended to be used as a lavicidal biocide for the control of mosquitoes in a variety of habitats. The target organisms intended to be controlled are 1st to early 4th larval stages of the genus *Aedes, Culex* and *Anopheles*.

The specific use is the control of mosquitoes in water such as:

* Flood water, roadside ditches, irrigation ditches, rice fields, pastures, woodland pools, snowmelt pools, standing ponds, standing pools, standing water containing mosquito larvae in fields growing crops such as alfalfa, almonds, asparagus, corn, cotton, dates, grapes, peaches and walnuts;
* Tidal water, salt marshes, catch basins, and storm water retention areas
* Polluted water (sewage lagoons, animal waste lagoons, etc.), water with moderate organic matter, and water with a high concentration of suspended solids.

Aquabac DF3000 is not intended to be used on treated, finished water reservoirs or drinking water receptacles when water is intended for human consumption.

The application rates recommended by the applicant are the following:

0.125 to 1 kg/ha, depending on the pollution of the water.

* Normal habitat: 0.125 to 0.500 kg/ha
* Polluted water/high population: 0.5 to 1.0 kg/ha

As there is 3000 ITU/mg potency in AQUABAC DF3000, this equates to 37 500 to 300 000 ITU/m2.

There is no specific dilution rate as long as the application is applied at 0.125 kg up to 1 kg/ha.

Suggested dilution rates are:

For ground application

* Back pack device: Dilution rate : 10 % - Mixture quantity per hectare : 15L;
* Portable power back pack: Dilution rate : 10 % - Mixture quantity per hectare : 10L-20L;
* Motorized overland application using an Airblast (pneumatic): Dilution rate : 2 % - 10 % - Mixture quantity per hectare : 12L - 100L;
* Other systems: Dilution rate : 1 % - 10 % - Mixture quantity per hectare : 15L (gun) - 300L (lance, spear)

For air application:

* Dilution rate: 6% (Low Volume) - 10% (Ultra-Low Volume) - Mixture quantity per hectare: 4L - 40L.

AQUABAC DF3000 is intended to be applied in conventional aerial and ground application equipment with sufficient water to provide thorough coverage of the target area. The amount of water needed depends on weather, type of spray equipment and mosquito habitat.

Ground applications should be done in 12-300 Litres of water per hectare in conventional equipment.

### Effects on target organisms and efficacy

The submitted studies to demonstrate efficacy of the product AQUABAC DF3000 according to the uses and doses claimed, are described below. These studies were carried out with the product AQUABAC DF3000 (43 % w/w Bti BMP 144).

1. **Laboratory test according to WHO method with the product AQUABAC DF3000 (43 % w/w *Bti* BMP 144) on 3rd and early 4th larval stages of *Aedes aegyti, Aedes albopictus, Culex pipiens and Anopheles gambiae*.**

A laboratory study was conducted to assess the efficacy of AQUABAC DF3000 applied in water and intended to control mosquito larvae. The trial is conducted by counting adult merging in artificially infested cups of water containing the product, in comparaison with an untreated control. The test method is adapted from WHO/CDC/WHOPES/GCDPP/2005.13. Only one dose is tested. The test was done on 100 third-early fourth larval stages for each species. Insects came from laboratory colony breedings. The trial is conducted separately for each species of mosquito. Mortality of the larvae and emerging adults were recorded after 24h, 48h and 7 days. The same procedure is applied to assess the residual activity of the product after 15 days. The efficacy is given by the pourcentage of reduction of emergence of adults between treated and untreated units.

AQUABAC DF3000, at a dilution of 1 % v/v in water and at an application rate of 10 g water mixture for 1 m² (i.e. 0.10 g AQUABAC DF3000 for 1 m²), showed 100 % efficacy after 24h. This efficacy lasted 15 days after application.

1. **Field test conducted in France with the product AQUABAC DF3000 (43 % w/w Bti BMP 144) according to an internal method on *Aedes caspius*.**

This experiment aims at evaluating plots, the larvicidal efficacy of AQUABAC® XT, AQUABAC® DF3000 and AQUABAC® 200G against the species *Ochlerotatus (Aedes) caspius*. The test took place on 16 and 17 April 2015 on a wetland located in France (Gard department).

The experimental apparatus includes an individual plot of 400 m2 per object. Within each plot are identified twenty sampling points. Just prior to treatment, observed abundances are relatively large, with an average over all plots, 36 larvae per sample **only composed of** **L2 stage**. Treatments are performed by **ground application** ( using a sprayer Laser® Industry 3610 (13 L) at constant pressure (2 bars), equipped with a suitable hydraulic nozzle air injection (anti-drift - Agricultural approval NTZ)) in the morning of 04/16/15. After 24 hrs, the observed abundances are very low with 1 larva by taking an average across all plots. The formulations of AQUABAC® DF3000, at a dose of **1.15 kg formulated product / ha**, achieved **98.1 %, efficiency in 24 hours**.

This study does not permit to conclude that the product is still effective after 15 days and that the product is also effective in a polluted environment.

1. **Semi-field test conducted with the product AQUABAC DF3000 (43 % w/w Bti BMP 144) according to an internal method on *Ochlerotatus (=Aedes) taeniorhynchus* and *Cx. quinquefasciatus*.**

AQUABAC® DF 3000 was applied to 2 manmade potholes. The potholes contained either 500 *Ochlerotatus* (=*Aedes) taeniorhynchus* or *Cx. Quinquefasciatus* mosquito. The potholes were a series of outdoor concrete basins, 6’ x 8’ external dimensions and 4’ x 5’ internal dimensions, with sloping sides and capacity of 60 US gallons. The potholes were filled with water and hog chow added for food (4 mg of hog chow for *Ochlerotatus* (=*Aedes) taeniorhynchus* and 10 mg for *Cx. quinquefasciatus*). AQUABAC® DF 3000 was diluted in1 litre of water to achieve a rate of 100 mg and applied to 2 manmade potholes with a sprinkling can. The number of live and dead larvae was evaluated the following morning (12 hours). The potholes treated with AQUABAC® DF 3000 had 100% mortality and the untreated control potholes had less than 2% mortality. But for the treated potholes, the percentage of recovery of the *Oc. taeniorhynchus* larvae was only 23 % (115 out of 500) and 9.4 % (47 out of 500) for the *Cx. quinquefasciatus* larvae. Furthermore, the test report was very succinct, there were no raw data and the tested dose is not very clear. So, results of this test are not considered relevant.

These field studies submitted demonstrate that the product AQUABAC DF3000 (43 % w/w Bti BMP 144) is efficient for ground application on mosquito’s species *Aedes (Ochlerotatus)* at L2 stage, at the minimum application rate of 1.15 kg/ha, instead of maximum 1 kg/ha claimed. According to the applicant, experimental conditions (variability of the speed of progress because of the heterogeneous nature of the ground) allow to explain why quantity spread is higher, and then a product application rate higher than claimed (+ 15%). Thus FR RMS considers that the claimed maximum application rate of 1kg/ha is acceptable as minimum application rate as the applicant has also submitted two semi-field trials (see below), one on *Cx. quinquefaciatus* and one on *Ae. albopictus*, at the claimed application rate of 1 kg/ha.

1. **Semi-Field test conducted in France with the product AQUABAC DF3000 (43 % w/w Bti BMP 144) on *Culex quinquefasciatus*.**

This experiment aims at evaluating the larvicidal efficacy of AQUABAC DF3000 against the species *Culex quinquefasciatus* according to the dose validated in the product assessment report and the summary product characteristics.

The semi-field test was carried out in 80 L LDPE bins. Fifty L2/L3 larvae were introduced into each bin 1 hour before treatment and successively at D3, D7, D10, D14 and D17. 72 hours after each introduction, Larvae were collected using a dip net and mortality was assessed. The operation was carried out until efficiency <50%. The formulation of AQUABAC DF3000, at a dose of 1 kg of formulated product / ha, achieved 99.2 % efficiency in 24 hours, 92.8% at D3, <90% until the end of the test.

The product AQUABAC DF3000 has shown a sufficient efficacy and can be used for the control of against larvae of *Culex spp*.

1. **Semi-Field test conducted in France with the product AQUABAC DF3000 (43 % w/w Bti BMP 144) on *Aedes albopictus*.**

This experiment aims at evaluating the larvicidal efficacy of AQUABAC DF3000 against the species *Aedes alobopictus* according to the dose validated in the product assessment report and the summary product characteristics.

The semi-field test was carried out in 80 L LDPE bins. Fifty L2/L3 larvae were introduced into each bin 1 hour before treatment. 48 and 96 hours after introduction, Larvae were collected using a dip net and mortality was assessed. The formulation of AQUABAC DF3000, at a dose of 1 kg of formulated product / ha, achieved 100 % efficiency in 48 hours. The product AQUABAC DF3000 has shown a sufficient efficacy and can be used for the control of against larvae of *Aedes spp*.

No field or semi-field studies have been submitted to support effectiveness for aerial application.

No field or semi-field studies have been performed on early 4th larval stage of *Aedes* and *Culex* and, 3rd and early 4th larval stages of *Anopheles* mosquitos. But a laboratory test shows effectiveness on third and early fourth instar larvae. So, efficacy against these developmental stages can be validated.

All relevant efficacy studies are presented in annex 6.

**Table 1: Summary of validated efficacy data**

|  |  |  |  |
| --- | --- | --- | --- |
| **Target Organisms** | **Rates and uses acceptable** | **Method of application** | **Time delay for the biocidal action** |
| Mosquito  *genus Culex*  *genus Aedes*  from 1st to early 4th larval stage | 1 kg/ha  waters with moderate organic matter | AQUABAC DF3000 may be applied using ground application hand-held equipment.  The product is used diluted in water | within 24 to 48 hours |

### Mode of action including time delay

*Bti* Strain BMP 144 is a Gram positive, spore forming rod-shaped bacterium that produces a crystalline protein inclusion which is toxic to larvae of some *Dipteran* insects upon ingestion.

The mode of action of *Bti* Strain BMP 144 results from toxic proteins contained in parasporal crystals. The crystals are taken up via ingestion and under the alkali conditions and protease present in the larvae gut, the crystal dissolves releasing the active protein delta endotoxins (Cry4Aa, Cry4Ba, Cry11Aa, Cyt1A) that induce disintegration of the larvae gut epithelium and consequent death of the larvae.

### Occurrence of resistance – resistance management / Unacceptable Effect[[3]](#footnote-3)

Concerning *Bacillus thuringiensis var. israelensis*, it was stated a resistance detected in a natural population of *Culex pipiens* of New York. However no other study confirmed this resistance and mechanisms were not characterized. To date, no other case of resistance was found in natural populations (CNEV, 2014).

In laboratory, three research teams selected mosquitoes strains *Aedes aegypti*, *Culex pipiens* and *Culex quinquefasciatus* in continuous contact with Bti during 20 to 30 generations and obtained only low levels of resistance to Bti (between 2 and 3 times). These researchers observed an increase of the DL50. However, this resistance disappears in some generations (3-4), when insects are replaced under normal conditions, i.e. without exposure to Bti and by allowing the reproduction with individuals coming from other lineages. This fast loss of resistance indicates the genetic instability of this one.

The absence of resistance of mosquitoes in spite of the massive use and for several decades of Bti is often explained by the fact that several toxins acts as a mixture, even in synergy; the selection of resistant populations to several toxins being less likely than to an only one. Indeed toxins Cry, even very close, can have different membrane receptors, making difficult a strong resistance to a combination of several toxins. Furthermore, the toxin Cyt is a key element of Bti, known to slow down the appearance of resistance to toxins Cry.

Finally, Bti presents a low residual activity (few days), thus can be qualified as having low environmental persistence. However, spores of Bti are found during several weeks to several months after a treatment. The bacteria being considered as few competitive compared with the other soil bacteria, the recycling of Bti is considered as unlikely.

Nevertheless, a number growing works shows that a residual activity of Bti can be found in certain conditions and that this activity could even, in some cases, be the consequence of a recycling of the spores of Bti, i.e seeding and multiplication of the bacteria. It is then the case in region Rhône-Alpes (France), where vegetable litters in decomposition, taken several months after an insecticidal treatment, showed a strong insecticidal activity against the larvae of mosquitoes. This toxicity is mainly due in fact to the presence of Bti, displayed by bacterial spreading and by the presence of coding genes for toxin of Bti. A laboratory strain of mosquito *Aedes aegypti* was selected during several generations with this " toxic litter " and allowed to obtain a resistance moderated to Bti but higher to toxin Cry considered separately. This resistant strain to Bti (strain " LITOX ") is the proof that Bti can, under an environmental persistent shape, lead to a resistance of mosquitoes.

So, Bti can, in certain conditions, produce crystals of toxins and lead to an increase of the resistance to Bti by the mosquitoes. The understanding of the mechanisms of persistence / recycling of Bti as well as the way mosquitoes resist is essential to aim towards a sustainable use of Bti and a better management of the resistance.

### Evaluation of the Label Claims

French competent authorities (FR eCA) assessed that :

The efficacy studies submitted by the applicant showed that the product AQUABAC DF3000 (43% w/w Bti) is effective against 1st to early 4th larval stages of *Aedes spp.* and *Culex spp.* at the application rate of 1kg of formulated product per hectare for ground application. In the absence of supporting data, the range of application rate claimed of 0.125 up to 1 kg/ha cannot be validated.

None of the submitted data permits to conclude on the effectiveness of AQUABAC DF3000 against *Aedes spp.* and *Culex spp.* for aerial application.

None of the submitted field or semi-field data permits to conclude on the effectiveness of AQUABAC DF3000 on early 4th larval stages of *Aedes spp and Culex spp.* for ground application. But a laboratory test shows effectiveness on early fourth larval stage.

None of the submitted data permits to conclude on the effectiveness of AQUABAC DF3000 against *Anopheles spp*. for ground and aerial applications. As efficacy is not proved on *Anopheles* genus, the claim against *Anopheles spp.* is not validated.

Finally, residual activity of 5, 7 and 15 days and effectiveness in polluted environments have not been proven in the field tests, these claims cannot be validated.

The product is effective within 24 to 48 hours.

### Summary of efficacy assessment

The efficacy level of the product Aquabac DF3000 (43 % w/w *Bti* BMP 144) is satisfactory for the uses proposed in the table below.

***Conditions of use linked to efficacy assessment***

As the risk of developing resistance to *Bti* cannot be excluded, the authorization holder should report any observed resistance incidents to the Competent Authorities (CA) or other appointed bodies involved in resistance management.

As with any insecticide a sound resistance monitoring and management plan is necessary to ensure *Bti* remains effective.

To ensure a satisfactory level of efficacy and avoid the development of resistance in susceptible insect populations, the following recommendations have to be implemented:

* Always read the label or leaflet before use and respect follow all the instructions provided.
* Adopt integrated pest management methods such as the combination of chemical, physical control methods and other public health measures, taking into account local specificities (climatic conditions, target species, conditions of use, etc).
* Alternate products containing active substances with a different mode of action, (to remove resistant individuals from the population).
* Do not [use/apply] the product in areas where resistance to the active substance (s) contained in this product is supected or established.
* Check the efficacy of the product on site : if needed, causes of reduced efficacy must be investigated to ensure that there is no resistance or to identify potential resistance.
* Equipment used for treatments must be appropriate, properly maintained and calibrated.
* Take into account the life cycle and characteristics of target insects to adapt treatments. In particular, target the most susceptible stage of the pest, timing of applications and areas to be treated.
* Inform the authorisation holder if the treatment is ineffective.
* Before treatment, determine precisely [the areas to be treated/routes to be followed] (on a map or a plan) .
* Spraying must not be carried out where there is upward air movement or where a temperature inversion prevents the spray cloud settling within the treated area.
* To optimise the treatment efficacy, do not apply in case of rain (forecasted for the next 24 hours) or wind (above 15 km/h).
* The product is not intended to be used water with high organic matter.

## Description of the intended use(s)

The biocidal product AQUABAC DF3000 is a larvicide (product TP18) used by professional operators, for the control of mosquito larvae in water habitats (irrigation ditches, reservoirs, lakes, rivers, rice field, canals, marshland, etc.). The product AQUABAC DF3000 should be applied using ground or aerial application equipment, diluted with quantities of water sufficient to provide uniform coverage of the target area.

Table: Summary of intended uses

|  |  |  |
| --- | --- | --- |
| MG/PT | Field of uses envisaged | Likely concentrations at which product will be used |
| Main Group 03; Pest Control  PT18:  insecticides, acaricides and products to control other arthropods | Professional uses | |
| Insecticide for use against larvae of mosquitoes | AQUABAC DF3000 (43 % w/w *Bti* BMP 144)  Mosquitoes larvae: 0.125 - 1 kg/ha |

## Risk assessment for human health

### Hazard potential

#### Toxicology of the active substance

As the active substance *Bacillus thuringiensis* *israelensis* strain BMP144 is technically equivalent to the active substance *Bacillus thuringiensis* *israelensis* serotype H-14 strain AM65-52 as included into Annex I of Directive 98/8/EC, the summary of information about the active substance is carried out with the data from the Assessment report of *Bacillus thuringiensis* subsp. *israelensis* serotype H-14 strain AM65-52 (CAS N° 68038-71-1)[[4]](#footnote-4).

Discussion in published literature indicates the highly species specific nature of the *Bti* δ-endotoxin, the lack of toxic effects in warm-blooded organisms and the lack of activation in the non-alkaline gut environment of mammals.

In a range of toxicological studies, completed using *Bti*, experimental infection of mice, rats, guinea pigs and rabbits was attempted by various routes. Single and repeat administration tests revealed an absence of acute or prolonged toxicity at doses of approximately 107 to 108 bacteria per animal. There were no indications of anaphylaxis in guinea pigs and repeated passage through mice induced no virulent response. Repeat administration of a dose in the order of 1011 or 1012 bacteria per rat/mouse for three weeks resulted in no pathogenicity. In none of these tests was there evidence of pathological symptoms, disease or mortality. Behavior and weight gain were unaffected by treatment and necropsy revealed no macroscopic effects. The re-isolation tests for various organs were negative. It was concluded that *Bti* was well tolerated by the test species used, showed no propensity to multiply within the host and was rapidly eliminated without causing adverse effects. *Bti* was confirmed to be innocuous.

The Medical Director responsible for the plant confirmed no abnormalities and no human health related or other adverse reactions to *Bti*.

*B. thuringiensis* may be responsible for opportunistic infections and the possibility of a human infection with *B. thuringiensis* is limited only to severe immunocompromised patients. There are no indications that Bti AM65-52 is involved in human pathogenicity, infectivity or toxicity.

The overall assessment of the acute toxicity/infectivity pathogenicity studies on *Bti* (AM65-52) indicates no evidence of toxicity/infectivity or pathogenicity for the human health.

*Bti* AM65-52 should be considered as a potential human sensitizer, at concentration above 5,0% w/v, as clearly demonstrate in a experimental test study on guinea pigs, according to the Buehler protocol.

The potential for *Bti* AM65-52 to cause adverse effects in humans is considered below.

Concerns in relation to bacteria and human health arise from two sources:

(1) A potential to cause infection in humans.

(2) A potential to cause a direct toxic effect.

The safety of *B. thuringiensis (Bt*) to mammals has been extensively evaluated with high levels of the entomopathogen administered by various parenteral or oral routes of exposure. There is no evidence to lead to a conclusion that the limited exposures following use of the biocidal product could result in a direct toxic effect in humans.

However, the *Bacillus* genus contains the virulent mammalian pathogen *B. anthracis*, and any assessment of *Bti* AM65-52 should include an assessment of the potential for the bacterium to cause infection in humans exposed to the biocidal product. Equally, the endotoxin produced by *Bti AM65-52* is immunologically similar to the enterotoxin produced by *B. cereus* which is known to cause diarrhoeal food poisoning. Nevertheless, the producer has shown that no enterotoxins are present in the manufactured product.

The ability of *Bti* AM65-52 to remain viable in mammalian tissue may lead to detection in humans, particularly in environments where the microbial agent is used for insect vector control.

In addition, the ubiquitous nature of *B. thuringiensis subsp. israelensis* (*Bti*) and its persistence has meant it has been identified as present in infections following traumatic wounding, although no confirmation that *Bti* has been causative in the infection process has been established.

There have been no reports of infective activity in cases where humans have been exposed directly (i.e. spraying preparations) to Bti. In terms of mammalian infection, the specific toxicity of the parasporal body is important because it is not activated in mammals. Clearance rates may be affected by the presence of vegetative forms in the inoculum. The toxicity of the alkali solubilised crystal δ-endotoxin of *Bti* is only relevant to the insect GI tract because it is not activated in the acidic conditions of the mammalian intestine. Therefore, the risk of *Bti* AM65-52 causing true infectious disease in mammals, including humans, is considered to be negligible.

Animal testing using a variety of conventional toxicity tests and a range of maximum challenge protocols has been completed to confirm that Bti has no adverse effects. Rats fed 2 x 1012 viable spores per kg bodyweight showed no adverse effects, and human volunteers were fed 3 x 109 spores per day for five consecutive days also without adverse effect (studies reported in 1959).

Bti entered the general circulation following s.c., i.p. or i.c. injection, and was detected in several tissues. The entomopathogen was rapidly cleared from the lungs of rats with no evidence of multiplication to indicate true infectivity. It was shown athymic mice were still capable of clearing the entomopathogen from the body and therefore an intact immune system was not required for successful clearance. However, athymic mice had higher levels in the spleen than euthymic mice.

Acute intratracheal instillation of *Bti* to rats at ca 108 CFU of ‘Vectobac’ technical material resulted in signs of toxicity during the first two days following dosing, but no evidence of pathogenicity or mortality. Acute intravenous administration to rats of ca 107 CFU resulted in no treatment related toxicity and no evidence of pathogenicity. This was also the case with mice dosed by intraperitoneal injection of 106, 107 or 108 CFU/g. No evidence for sub-acute toxicity of *Bti* AM65-52 was found in the dog dosed at ca 106 Bti spores/mL for 90 days and there were no indications of treatment-related toxicity among rats dosed for 14 days by inhalation exposure at up to 1.84 x 106 spores/L air/day.

Cell culture studies are required for viruses and viroids or specific bacteria and protozoa with intracellular replication. This is not applicable to *Bti* AM65-52 which does not replicate in warm-blooded organisms.

The Bti δ-endotoxin consists of a four protein complex and is specifically toxic to insects, as it requires a very high pH 10 for activation. The lack of toxic effects in warm-blooded organisms and the lack of activation in the non-alkaline gut environments of mammals results in no adverse effect of the material in the context of human health. An assessment of the health effects of Bti on operators involved in the fermentation process and other staff likely to be exposed to the material confirmed no abnormalities and no human health related or other adverse reactions to *Bti*. However the specific exposure conditions are not representative for the Vectobac proposed uses.

An investigation into human infection by the Bacillus genus within the confines of a hospital looked at *Bt* presence in post-trauma infection. While the study concluded the presence of *Bt* did not constitute transient bacteraemia, it recognised that strain definition and strain pathogenicity are vital factors in the disease evaluation process. The study concluded that *Bti* AM65-52 is not implicated as a causative agent in human infection.

A study was presented to investigate the hypersensitivity potential of the technical powder product, ‘Vectobac’, based on *Bti* AM65-52, using the Buehler method. The results of this study indicate that Technical Powder VectoBac (Code 43494) administered as a 50% w/v formulation in distilled water during induction and as a 5% w/v formulation in distilled water during primary challenge, does produce dermal sensitization in the guinea pig. The formulated product VectoBac WG, under a Maximization test, was not considered a sensitizer.

Several Bt products including Vectobac WG have been in use for several decades, according to the manufacturer, with no severe findings reported. However data show that some adverse effects occur following direct human exposure especially during and after spraying in general population, but the amount and the relevance of the symptoms observed were not coherent among studies. Many of the effects observed were related to respiratory distress as well as skin reactions supporting the hypothesis that the exposure to commercial products based on Bt could possibly lead to sensitization/allergenicity reactions.

A study on humans showed that after exposure it is possible to observe vegetative *Bti* AM65-52 presence in samples, followed by clearance (which occurs after several days or weeks), without acute adverse effects.

In conclusion according to the data submitted, regarding the risk poses to human by *Bti* AM65- 52:

1. Pathogenicity and infectivity potential: there is no evidence that *Bti* AM65-52could lead to infections in humans, so it has to be considered safe with the precautionary exception to prevent the exposure of immunosuppressed subjects which must be considered at risk;

2. Direct toxic effects: There is evidence that *Bti* AM65-52 technical powder could induce sensitization in animal model. Human data are not conclusive as well as epidemiological records from spray campaigns. On this basis the risk of sensitization and / or allergenicity in human cannot be excluded and therefore ‘*Bti* AM65-52 should be considered as a potential human sensitizer. Thus the product should be labelled with safety phrases such as avoid contact with skin, wear gloves when handling the product, do not breath dust. It should not be labelled with the risk phrase Xi on the basis that the guideline studies do not show this product to be a sensitizer.

#### Toxicology of the substance(s) of concern

Considering the definition of a substance of concern set in the Guidance on the BPR Volume III Humana Health – Part B Risk Assessment (updated Version 4.0, December 2017), the product AQUABAC DF 3000 contains **Sodium Dibutylnaphthalene sulphonate** (at 10%w/w) as a substance of concern (band A). Conclusions remain unchanged: please see section “risk assessment for human health” for local risk assessment (Mutual recognition in sequence – May 2018).

#### Toxicology of the biocidal product

The toxicology of the biocidal product was examined appropriately according to standard requirements. The product was not the representative product in the EU- review program for inclusion of the active substance in Annex I of Directive 98/8/EC.

The basis for the health assessment of the biocidal product is laid out in Annex 5 ”Toxicology – biocidal product”

No study has been provided.

##### Percutaneous absorption

Not relevant for micro-organisms.

##### Acute toxicity

There is no additional information on the acute toxicity of the product AQUABAC DF 3000 containing *Bacillus thuringiensis subsp. israelensis*. The classification of the product is established by calculation.

##### Irritation and corrosivity

There is no additional information on the irritation toxicity of the product AQUABAC DF 3000 containing *Bacillus thuringiensis subsp. israelensis*. The classification of the product is established by calculation.

##### Sensitisation

There is no additional information on the irritation toxicity of the product containing *Bacillus thuringiensis subsp. israelensis*. The classification of the product is established by calculation.

While micro-organisms should be considered as potential sensitisers, the current labelling legislation is only directly attributable to chemicals. Furthermore, current available studies for skin sensitisation assessment are not appropriate for micro-organisms. Consequently products containing microbials are required to carry a precautionary phrase but are not labelled H317 unless the sensitisation response can unequivocally be attributed to a specific chemical co-formulant. Product does not contain sensitising co-formulant. Therefore, the product is not classified as H317. However, since the product contains a micro-organism, AQUABAC DF 3000 should carry the default precautionary statement: “Contains *Bacillus thuringiensis israelensis*, micro-organisms may have a potential to provoke sensitising reactions”.

##### Other studies

None

### Human exposure assessment

AQUABAC DF 3000 contains 43 % w/w of *Bti* strain BMP 144.

#### Identification of main paths of human exposure towards active substance from its use in biocidal product

Aquabac DF 3000 is applied onto mosquito natural habitat by professional workers. The product will be applied at a concentration of 0.125 up to 1 kg/ha.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Exposure path** | **Industrial use** | **Professional use** | **General public** | ***via* the environment** |
| Inhalation | Not applicable | Yes | Yes (indirect) | Negligible |
| Dermal | Not applicable | Yes | Yes (indirect) | Negligible |
| Oral | Not applicable | Negligible | Negligible (indirect) | Negligible |

#### Direct exposure as a result of use of the active substance in biocidal product

##### Exposure of professional users

No models are currently available to estimate professional exposure from the application of micro-organisms. Furthermore, the derivation of reference values was not considered needed since the microorganism was not shown to be toxic, pathogenic or infective based on the available data and studies.

Therefore, a quantitative estimation of professional exposure is not necessary and the exposure has only been qualitatively estimated.

The product is intended to be applied by ground (by convential ground equipment) or aerial equipment (spraying or granule spreader).

The typical routes of exposure are from dermal absorption, inhalation and ingestion. The potential for systemic exposure from dermal absorption does not need to be considered because Bti is not supposed to penetrate intact skin. The potential routes of exposure are therefore from inhalation or ingestion. Ingestion can only occur as secondary to inhalation and is thus considered negligible. Inhalation exposure can occur during mixing and loading and during spraying. In case of enclosed vehicle or aircraft cabs, the exposure during application is considered negligible.

Due to the potential of all micro-organisms being potential sensitizers, suitable protective clothing and equipment must be considered: protective gloves, working coverall, goggles and respiratoy mask (with P3 filter) during all phases with potential exposures.

Overall, it is concluded that under normal conditions of use and when the label recommendations quoted above are well followed, the risk for professionals is low. However, AQUABAC DF 3000 should not be used by professional workers affected by immunodeficiency, primary or secondary, or in treatment with immunosuppressive agents, which can significantly reduce the effectiveness of the immune system response.

##### Exposure of non-professional users

Not relevant, the product is for professionnal users only.

#### Indirect exposure as a result of use of the active substance in biocidal product

Ground spray application could lead to an exposure to the spray drift, if a bystander is walking next to an area being treated. Bystanders are excluded from treated areas to ensure only protected professionals can possibly be exposed to AQUABAC DF 3000. The risk is thus considered minimal.

In order to reduce exposure of residents and to be consistent with a French order applying to aerial spray of phytopharmaceutical products, a drift buffer zone of 50 m should be respected for AQUABAC DF 3000 as biocidal product applied with an aerial equipment.

Finally, in case of early re-entry after treatment, it is recommended to workers to wear a coverall and gloves.

#### Combined exposure

Not relevant.

### Risk assessment for human health

#### Risk for direct exposure

##### Professional users

The derivation of reference values was not considered needed since the microorganism was not shown to be toxic, pathogenic or infective. No exposure models are currently available to estimate human exposure from the application of micro-organisms.

According to Guidance on the BPR (Volume III Parts B+C, v4.0, December 2017), risk caracterisation for local effects should be done. Hazard is categorised as “low” regarding the classification (Eye irrit. Cat 2, H319) therefore the following is proposed in order to conclude qualitatively on the acceptability for professional exposure:

|  |  |  |  |
| --- | --- | --- | --- |
| **Hazard effects** | **Frequency and duration of potential exposure** | **Degree of potential exposure under best practice conditions** | **Relevant RMM or PPE** |
| Eye irrit. Cat 2,  H319 | More than few minutes but equal to or less than few hours per day | Controlled exposure, spraying application, and PPE worn | Chemical goggles |

In order to minimise exposure or possible health effects, goggles must be worn by profesionnals.

Considering the definition of a substance of concern set in the Guidance on the BPR Volume III Humana Health – Part B Risk Assessment (updated Version 4.0, December 2017), the product AQUABAC DF 3000 contains **Sodium Dibutylnaphthalene sulphonate** (at 10%w/w) as a substance of concern (band A). Conclusions remain unchanged: in order to minimise exposure or possible health effects, goggles must be worn by profesionnals.

The results of the attrition and resistance of the granules and the wet sieve test are outside the acceptable limits (see 2.1.2.2 physico-chemical properties), therefore no data is available the particles generated from attrition and the particle size. Professionals may be exposed during mixing and loading of particles < 50 µm. A respirable mask (with P3 filter) is recommended for microorganism during the mixing and loading phase.

Considering the intended uses and the recommended PPE, the risk for professionals is considered acceptable. However, AQUABAC DF 3000 should not be used by professional workers affected by immunodeficiency, primary or secondary, or in treatment with immunosuppressive agents, which can significantly reduce the effectiveness of the immune system response.

##### Non-professional users

Not relevant.

#### Risk for indirect exposure

Following the above given reasons for abstaining from an estimation of professionals risk assessment, this also applies with regard to bystanders. With regard to the application method, bystander is supposed to be negligible for spray drift during application. Bystanders are excluded from treated areas to ensure only protected professionals can possibly be exposed to AQUABAC DF3000.The risk is thus considered minimal.

Finally, in case of early re-entry after treatment, it is recommended to workers to wear a coverall and gloves.

#### Risk for indirect exposure via residues and food

No specific residue data were submitted in the context of this dossier. The product AQUABAC DF 3000 is intended to be applied by professional users, outdoor on flood water, roadside ditches, irrigation ditches, floodwater, rice fields, pastures, woodland pools, snowmelt pools, standing pools, tidal water, salt marshes, catch basins, storm water retention areas, standing water in fields growing crops (such as alfalfa, almonds, asparagus, corn, cotton, dates, grapes, peaches, and walnuts), sewage lagoons and animal waste lagoons.

Intended uses on rice fields and standing water in fields growing crops could lead to an exposure via food consumption. Therefore, only these uses will be discussed in the residue section. No data on potential exposure have been submitted.

As regards the use in water irrigating rice, AQUABAC DF3000 will only be applied in presence of water when mosquitoes proliferate and towards the end of the rice growing period the fields are dried approximately 4 weeks before the grain harvest. Rice grains are also covered by a husk that is removed prior to consumption.FR is of the opinion that the use in water irrigating rice is acceptable with a pre harvest interval of 1 month.

Concerning irrigation water (except water surrounding rice) it is not possible to predict their fate after treatment. Time between product application and crop irrigation may vary and all types of crops can be irrigated with this treated water (at any growth stage). Therefore, AQUABAC DF3000 should not be applied in water when irrigation water is intended to be used on food crops (except rice).

As regards standing water, as no data or justification has been given, application of AQUABAC DF3000 should not take place when edible parts of plants are present.

In Annex 5 “Residue behaviour”, the results of the residue assessment are laid out. Open literature data were considered.

#### Risk for consumer via residues and food

Based on the intended uses and the proposed restriction, the acute or chronic exposure to residues in food resulting from the intended uses is unlikely to cause a dietary risk to consumers. AQUABAC DF 3000 should not be applied in waters in irrigated crops, except in water irrigating rice for which a pre harvest interval of 1 month is required. Regarding consumer health protection, there are no objections against the intended uses. As regards, treatment in standing water in fields growing crops, AQUABAC DF 3000 should not be applied when edible parts of plants are present.

#### Risk for combined exposure

Not applicable.

#### Summary of risks characterisation of the product for human health

No unacceptable risk has been identified for professionals using AQUABAC DF 3000 with ground or aerial equipment when appropriate PPE are worn during mixing/loading and application.

For indirect exposure during ground application, the risk is considered acceptable for bystanders since they are excluded from treated areas. After aerial application, the risk for residents is considered low if drift buffer zone of 50 m is respected.

For workers, the risk is considered acceptable if a coverall and gloves are worn in case of early re-entry after treatment.

***Risk mitigation measures linked to risk assessment human health***

* Professionals must wear gloves, working coverall, goggles and respiratory mask (with P3 filter) during mixing/loading and application phases.
* Since *Bacillus thuringiensis israelensis* may be responsible of opportunist infection in sever immunocompromised people, the product should not be used by subjects affected by immunodeficiency or in treatment with immunosuppressive agents.
* Non users are not permitted in area being treated.
* In case of re-entry after treatment, it is recommended to workers to wear a working coverall and gloves.
* When used in water irrigating rice, a pre harvest interval of 1 month is required
* Do not apply in standing water in fields growing crops when edible parts of plants are present (except rice).

## Risk assessment for the environment

As the active substance *Bacillus thuringiensis* *israelensis* strain BMP144 is technically equivalent to the active substance *Bacillus thuringiensis* *israelensis* serotype H-14 strain AM65-52 as included into Annex I of Directive 98/8/EC, the summary of information about the active substance is carried out with the data from the Assessment report of *Bacillus thuringiensis* subsp. *israelensis* serotype H-14 strain AM65-52 (CAS N° 68038-71-1)[[5]](#footnote-5).

Considering the definition of a substance of concern set in Guidance on the Biocial Products Regulation (vol IV B+C)[[6]](#footnote-6)[1], AQUABAC DF 3000 does not contain any substance of concern for the environment.

### Fate and distribution in the environment of *Bacillus thuringiensis* subsp. *israelensis* Serotype H-14 Strain AM65-52

#### Degradation

##### Abiotic degradation

###### Hydrolysis in function of pH

Not applicable.

###### Photolysis in water

Not applicable.

###### Photolysis in soil

Not applicable.

###### Photodegradation in air

Not applicable.

##### Biotic degradation

###### Aquatic compartment

* Ready biodegradation / inherent biodegradation

Not applicable.

* Degradation in water/sediment system

The half life of spore in water is derived from a laboratory study carried out with *Btk* in four types of water: filtered-distilled, tap, lake and sea. In distilled and tap water, approximately 50 % of the original cell population died off rather rapidly during the first 20 days following inoculation. *Btk* was found to be far more persistent in fresh water than in sea water, generally considered bactericidal to non-marine bacteria (Pramer et al., 1963, cited in Menon and De Mestral, 1985). The highest half life (approximately 50d) was observed in lake water which contains a higher concentration of available nutrients favourable to *Btk* survival. Therefore an half life of 50 days is assumed for the spore of *Bti* in water.

Several studies that have investigated the persistence of *Bti* in water (Mulla et al., 1985; Beehler et al., 1991; Hougard et al., 1995; all cited in Glare and O’Callaghan, 2000), and showed that larvicidal activity of Bti disappears within 1-4 weeks. An average dissipation half-life of 14 days in water has been used for the biological activity of the toxins of *Bti* strain AM65-52.

Microcosm studies have shown that suspended particles in water greatly reduce the activity of *Bti* products towards mosquito larvae, but have no discernable effect on the number of viable bacteria. Disappearance of larvicidal activity is attributed to the adsorption of the insecticidal toxins and vegetative cells to sediment particles. However, adsorption was reversible with mechanical stirring (W. Sheeran and Fisher, 1992 cited in Glare and O’Callaghan, 2000).

* Other degradation pathway (ex. Seawater...)

See Degradation in water/sediment system.

###### Degradation in STP

No data on degradation in STP.

###### Terrestrial compartment

* Aerobic degradation

Experimentally determined half-lives for spores in soil are usually in the range of 100-200 days (Hansen et al., 1996). In a field soil, Pedersen et al. (1995) found a long-term persistency of *Bt*k DMU67R spores, with a half-life of 120 days, and this value has been selected for the half life of spore of Bti in soil.

The persistence of protein-crystals, assessed by bioassay of insecticidal activity, is shown to fall rapidly in soil, as a consequence of degradation by microorganisms and adsorption onto soil particles. The insecticidal activity half-life has been calculated (West, 1984) in the range 2.7-5.2 days, in absence and following the addition of an organic supplement, respectively. The worst case half life of 5.2 days has been chosen for the exposure assessment.

* Anaerobic degradation

Not relevant

#### Distribution

*Bacillus thuringiensis* (*Bt*) has been isolated worldwide from a range of habitats. In soil, the number of *Bt* spores has been found to vary between less than 2x102 to 5x104/g soil (P.A.W. Martin, 1991). As a general figure, the occurrence of *B.thuringiensis* subsp. *israelensis* (*Bti*) in soil accounts for about 20% of *Bt* serotypes (Martin and Travers, 1989). Pedo climatic conditions are likely to affect persistence, e.g. organic matter content, pH, soil texture, solar radiation etc. Although *Bt* bacteria generally represent an indigenous part of the soil microbiota community (De Respinis *et al*., 2006; Vettori et al., 2003) they do not compete aggressively with other soil micro-organisms (West et al., 1984; Akiba, 1986) and, as result of degradation of vegetative cells and poor germination of spores, are not adapted to survive as “active” members of the soil microbial community. For instance, the growth of *Bt* subsp. *aizawai* has been observed by West et al. (1985) but only when soil has been supplemented with nutrients or sterilized. The low capacity of *Bacillus thuringiensis* spores to germinate in soil restricts population growth and no epizootics with *Bacillus thuringiensis* subsp. *israelensis* have ever been reported.

Because of adsorption of spores protoxins and toxin on soil (Venkateswerlu and Stotzky 1992, summarized in Goodyear, 2005; Tapp and Stotzky, 1995; Crecchio and Stotzky, 1998; Crecchio and Stotzky, 2001), no leaching to the groundwater is expected. *Bt* has been shown to not migrate in soil under artificially and naturally irrigated conditions (Akiba, 1991, in Goodyear, 2005). However, no Koc has been experimentally determined and a values of KOC = 1000 L/kg is assumed for adsorption, in order to determine exposure assessment.

#### Accumulation

Not applicable.

#### Behaviour in air

A rapid degradation in air is assumed since inactivation by solar radiation is a very important factor causing loss of activity and degradation of bacteria spores and δ-endotoxin crystals in the field environment (Griego and Spence, 1978 Myasnik et al., 2001; Pusztai et al., 1991). Such degradation has been shown in an aerial spray program, where the *Btk* concentrations in the air showed an initial half-life (10-hour period from start of spraying) of 3.3 hours. The overall half-life determined during the nine-day monitoring period was 2.4 days, (Teschke *et al.*, 2001).

### Effects on environmental organisms for *Bacillus thuringiensis* subsp. *israelensis* Serotype H-14 Strain AM65-52

#### Aquatic compartment (including water, sediment and STP)

##### Aquatic organisms

The table below summarizes all the data available in the CAR for *Bacillus thuringiensis* subsp. *israelensis* Serotype H-14 Strain AM65-52 in aquatic compartment.

Table 1: summary of all the data available for *Bacillus thuringiensis* subsp. *israelensis* Serotype H-14 Strain AM65-52 in aquatic compartment

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Test item | Species | Endpoints | Toxicity | Reference |
| Btia | *Onchorhynchus mykiss* | LC50 – 96h | >370 mg/L | IIIA,  8.2.1-01 |
| Btia | *Lepomis macrochirus* | LC50 – 96h | >600 mg/L | IIIA,  8.2.1-02 |
| VECTOBAC technicalb | *Onchorhynchus mykiss* | NOED – 32d | no adverse effects  water: 1.1x1010 CFU/L; 3.7x105 ITU/L);  diet: 1.72x1010 CFU/g ; 5.7x105 ITU/g);  fish growth in the VECTOBAC treatment significantly lower than in control, due to high turbidity and suspended solids | IIIA,  8.2.1-03 |
| VECTOBAC technicalb | *Lepomis macrochirus* | NOED – 30d | no adverse effects  water: 1.2x1010 CFU/L ; 4x105 ITU/L;  diet: 1.31x1010 CFU/g; 4.4x105 ITU/g | IIIA,  8.2.1-04 |
| VECTOBAC technicalb | *Cyprinodon variegatus* | NOED – 30d | no adverse effects  water: 1.3x1010 CFU/L; 4.3x105 ITU/L;  diet: 2.1x1010 CFU/g; 7x105 ITU/g | IIIA,  8.2.1-05 |
| VECTOBAC technicalc | *Daphnia magna* | NOEC – 10d | >50 mg /L (1x1010 CFU/L) | IIIA,  8.2.2-01 |
| VECTOBAC technicalb | *Daphnia magna* | NOEC – 21d | 0.5 mg/L (1x108 CFU/L; 3.3x103 ITU/L) | IIIA,  8.2.2-02 |
| VECTOBAC technicalb | Grass shrimp (*Palaemonetes vulgaris)* | NOEC – 31d | 2.0 x 1010 CFU/(6.6 x 105 ITU/) | IIIA,  8.2.2-03 |
| VECTOBAC technicald | Mayfly nymphs (*Hexagenia* sp) | NOEC – 18d | 2.0 x 1010 CFU/L | IIIA,  8.2.2-04 |
| VECTOBAC technicalb | *Amphiascus minutus* | NOEC – 10d | 50 mg/kg (1x10 10 CFU/g (3.3x105 ITU/g) | IIIA,  8.2.2-05 |
| Toxins from Bti (25 – 130 kDa) | *Euglena ssp.; Chlamydomonas sp.; Oedogonium sp.; mixed algal cultures; Oscillatoria sp.* (cyanobacterium) | n.a. | no adverse effects |  |

**a** no CFU nor ITU content was indicated

**b** VECTOBAC Technical used in toxicity bioassays had a biopotency of 2x1011 CFU/g Bti and 6.6x103 ITU/mg Bti

**c** VECTOBAC Technical used in bioassays had a biopotency of 7.2x1010 CFU/g Bti; no ITU content was indicated

**d** VECTOBAC Technical used in bioassays had a biopotency of 2.0x1010 CFU/g Bti; no ITU content was indicated

**Additional endpoints:**

In addition to these laboratory studies, two field studies (IIIA 8.2.2-06 and IIIA 8.2.2-07 were presented in the active substance dossier, and these studies showed no adverse effect on non target species following repeated treatments with *Bti*.

However, a study by Hershey *et al*, 1998 in Minnesota wetlands showed that after three years of VECTOBAC applications the number of non dipteran predators was affected, so that the need for long-term data to evaluate food web effects was expressed. Also Pont et al., 1999; observed some negative effects on repeated treatment with Bti but with higher doses than those intended in the product authorization dossiers. These two studies have been briefly reported in the table 2. On the opposite, other papers showed the lack of negative impact on treated ecosystems (Balcer et al., 1999; Schmude et al., 1999; Becker, 2005; Lacey & Merritt, 2002; Lacey, 2007 and, more recently, Lundstrom et al., 2009 (see assessment report for more information), so that there are not unambiguous evidences on this issue.

An additional bibliographical review, mainly based on studies carried out in Europe has been performed to investigate if long term repeated applications, which are expected in France for sanitary purpose, are covered by the available studies. French laboratory reports with not published results have also been taken into account (Franquet et al. (no year); Le Goff et al., 2009; Roucaute et al., 2013). For comparison purposes, the intended doses for AQUABAC DF3000 are 5.00E+08 ITU/ha to 4.0E+09 ITU/ha. A maximum of 8 applications are intended in the CAR for this active substance. Several studies describe difficulties to interpret the observation because of the high influence of climatic conditions which are very variable amongst the monitoring. As seen in the table 2, only few adverse effects on insects are reported in the European studies. Nevertheless, it should be noted that quite all these studies have been carried out with only one or two applications or with lower doses than those intended in the product authorization dossiers. Therefore, these studies do not allow to excluding adverse effects on aquatic organisms for 8 applications at the highest intended doses. Additionally, only few studies are dealing with the impact of the food chain. Two studies carried out in Germany showed that mosquitoes are only a small proportion of birds diet. A study in the south of France reported differences in food of house martins between treated and control site, and a consecutive decrease in chicken per nest in treated area. Nevertheless, in this study climatic conditions were not monitored and variations of water level could have had a strong impact on invertebrate dynamics. Thus, potential impacts of Bti treatment on food chain appears as not elucidated.

Table 2: Key information from the bibliographical studies monitoring biodiversity in area treated with *Bti*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Study | Location | Duration of monitoring | Product, dose, and number of applications carried out in the studies | Observed effect |
| Hershey et al., 1998 | Minnesota (USA) | 3 years | 2.4E+09 ITU/ha, 6 applications /year | Low effect on first year. Decrease of 60% of total insect density, highest decrease in density observed for Diptera, including Nematocera and Brachycera, and in a lesser extend for Coleoptera. Decrease in total insect richness. |
| Pont et al., 1999 | South of France | 12 days | 2.4E+09 ITU/ha  4.8E+09 ITU/ha  9.6E+09 ITU/ha, one application | 2L/ha: the density of chironomids significantly lower in the treated area at 5 days. No observed effect at 2 and 8 days. No significant effect on emergence  4L/ha: No significant effect on emergence. Changes in community structure  8L/ha: significant decrease (62-88%) of emergence. Changes in community structure |
| Franquet et Fayolle (no year) | South of France | 2-3 years | 1.8E+09, 3.6E+09, 9.6E+09 ITU/ha, one application | Modification of population dynamic for one year for the highest dose |
| Le Goff et al., 2009 | West of France | 2 years | No information dealing with the doses per hectare  5-10 applications per year | No observed effect of treatment on invertebrate. High impact of climatic conditions |
| Lagadic et al., 2013 | West of France | 7 years | 6.0E+08 – 9.0E+08 ITU/ha, 7-8/ year | No observed effect on invertebrate |
| Roucaute et al., 2013 | 4 regions in France, including Center and South of France, Corsica and French Guiana | 2 years | 1E+09 ITU/ha, 1-2/ year | No observed effect, high impact of climatic conditions |
| Caquet et al. 2011 | West of France | 2 years | 6.0E+08 ITU/ha and 1.2E+09 ITU/ha, 5-6/year | High impact of climatic conditions. Bti treatment increased abundance of Chironomini and Orthocladiinae larvae. No effect on *Nereis diversicolor*, *Coropjium volutator* and midge larvae. Zone treated with 6.0E+08 ITU/ha was treated for 7-8 years before the study (5-8 applications/year)  Additional monitoring for two years (not published data): No effect on taxonomic richness, Shannon’s diversity index and Pielou’s evenness |
| Duchet et al., 2008 | South of France  (microcosms in shallow marsh) | 21 d | 9.6E+08 and 3.0E+09 ITU/ha, 1 application | No effect on abundance of *Daphnia pulex*, but at 21d, there are less significantly fewer younger daphnids and more older daphnids in microcosms treated with Bti than in control. |
| Duchet et al., 2010 a | South of France  (microcosms in shallow marsh) | 21 d | 9.6E+08 and 3.0E+09 ITU/ha, 1 application | No effect on density of *Daphnia magna*, except at 21d with the highest Bti concentration, which induced a significant negative effect on daphnids density. |
| Duchet et al., 2010 b | South and west of France (microcosms in shallow marsh) | 14j | 3.0E+09 ITU/ha, , 1 application | No effect on density of *Daphnia magna* and *Daphnia pulex* |
| Persson Vinnersten et al., 2010 | Sweden | 6 years | 2.6E+09 - 3.0E+09 ITU/ha, no information dealing with the number of applications | No effect on invertebrates |
| Lundström et al., 2010 a and b | Sweden | 6 years | 3.0E+09 ITU/ha, twice the first year, once the second year, once the fourth and the fifth year | No effect on Chironomidae production, only small effects on chironomid species richness but tendency to increase the colonization-extinction dynamics |
| Becker 1998 and Becker 2003 | Germany | Several years? | 2.5E+09 ITU/ha. Number of applications not provided | No detailed results. No impact on insect, *Aedes* mosquito is only a minor part of the food of birds |
| Poulin et al., 2010 | South of France | 3 years | 3.21E+09 ITU/ha, more than one application (no detailed information) | Intake of Nematocera by house martins divided by 3 at treated site, whereas intake of ants increased (70%). Decrease in number of chicks produced per net in treated area (2.3 versus 3.2 in control area). |

Hershey, A. E., A. R. Lima, G. J. Niemi and R. R. Regal, 1998. Effects of *Bacillus thuringiensis israelensis* (*Bti*) and methoprene on nontarget macroinvertebrates in Minnesota wetlands. Ecological Applications. 8 : 41-60

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Franquet E., Fayolle S., Cazaubon A. Etude d’impact d’un éventuel traitment au Bti sur le territoire du Parc naturel régional de Camargue. 156p.

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[**Lagadic, L.**](http://www.scopus.com/authid/detail.url?authorId=56039721700&amp;eid=2-s2.0-84892550076)http://www.scopus.com/static/images/s.gif, [**Roucaute, M.**](http://www.scopus.com/authid/detail.url?authorId=16550832800&amp;eid=2-s2.0-84892550076), [**Caquet, T.**](http://www.scopus.com/authid/detail.url?authorId=6603409732&amp;eid=2-s2.0-84892550076)**, 2014** Bti sprays do not adversely affect non-target aquatic Invertebrates in French Atlantic coastal wetlands[**Journal of Applied Ecology**](http://www.scopus.com/source/sourceInfo.url?sourceId=29547&origin=recordpage) Volume 51, Issue 1, February 2014, Pages 102-113

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Justification of PNECwater

The PNEC and PNED for surface water are derived in the Annex 1 CAR using the NOEC of 0.5 mg/L (corresponding to 1 x108 CFU/L and 3.3x103 ITU/L) obtained in a Daphnia reproduction test. An assessment factor of 10 was applied to this to give a PNEC of 0.05 mg/L corresponding to PNEDsurface water =1 x107 CFU/L and PNECsurface water =3.3x102 ITU/L.

##### Sediment dwelling organisms

No data dealing with the toxicity of *Bacillus thuringiensis* subsp. *israelensis* Serotype H-14 Strain AM65-52 on sediment have been provided in the Annex I CAR nor in the AQUABAC DF 3000 dossier. However, several papers show contradictory results about the toxicity of *Bacillus thuringiensis* subsp. *Israelensis* onpredators of targeted organisms and the effects arising from long term and large scale use of AQUABAC DF 3000 on natural biological diversity should be assessed.

##### STP micro-organisms

Additional endpoints: A study was conducted using toxins from *Bti* (25 – 130 kDa), tests were performed using *Bacillus megaterium*, *B. subtilis*, *B. cereus*, *Staphylococcus faecalis*, or *S. aureus*. The overall conclusion of the tests was that no bacteriostatic or bactericidal activity was detected in the dilution or disk-diffusion assays with the toxins from *Bti* against the various pure and mixed cultures regardless of whether the cultures were incubated under starvation or non-starvation conditions.

Recently Mizuki *et al*. (2001) recovered at high frequency *Bt* from activated-sludge system environments in an urban sewage-digestive plant, and the highest density was 1.6 x 103 CFU/ml. Additionally, no antibiotic activity of the Insecticidal Crystal Proteins (ICPs) from *Bti* against a variety of gram-positive bacteria was observed.

Justification of PNECmicororganisms

There is no expectation that the use of AQUABAC DF 3000 will have an adverse effect on the microbial activity occurring in sewage treatment plants and no PNECmicroorganisms has been therefore derived.

#### Atmosphere

No data.

#### Terrestrial compartment

The table below summarises all the data available for *Bacillus thuringiensis* subsp. *israelensis*

Serotype H-14 Strain AM65-52 in terrestrial compartment.

|  |  |  |  |
| --- | --- | --- | --- |
| Species | Type of test | Endpoint(mg/kgdry soil) | Reference |
| Soil microorganisms | Exposure of *Bacillus megaterium*, *B. subtilis*, *B. cereus*, *Staphylococcus faecalis S. aureus* to Bti toxin | No observed effect | III A, 8.2.3-01 |
| Earthwormsa | LC50 - 30 day | 1000 mg/kg dry weight soil  (4.8x1010 CFU/kg dw soil; 8x106 ITU/kg dw soil) | III A, 8.5-01 |
| Plants | No test carried out |  |  |
| Bird (Mallard duck) a | 5‑day LD50 | >3077 mg/kg bw day (6.2x1011 CFU/kg bw day ; 2.03x107 ITU/kg bw day) | IIIA, 8.1-01 |
| NOEC | 3077 mg/kg bw day (6.2x1011 CFU/kg bw day ; 2.03x107 ITU/kg bw day) |  |
| Bird (Northern bobwhite) a | 5‑day LD50 | >3077 mg/kg bw day (6.2x1011 CFU/kg bw day ; 2.03x107 ITU/kg bw day) | IIIA, 8.1-02 |
| NOEC | 3077 mg/kg bw day (6.2x1011 CFU/kg bw day ; 2.03x107 ITU/kg bw day) |  |
| Bee a | 14-day oral toxicity, LC50 | >0.124 mg /bee/day (2.5x107 CFU/bee/day; 8.2x102 ITU/bee/day) | IIIA, 8.3-01 |
| Bee b | 48-h toxicity, LC50 | Contact toxicity:  LD50 >100 μg (1.8x106 CFU; 3x102ITU) /bee  Oral toxicity :  LD50 > 108.4 μg (1.9x106 CFU; 3.2x102 ITU)/bee | IIIB, 10.3-01 |

a VECTOBAC Technical used in bioassays had a biopotency of 2x1011 CFU/g Bti and 6.6x103 ITU/ mg Bti

bTest carried out with VECTOBAC WG, provided for the Annex 1 CAR.

Additional endpoints: No data.

Justification of PNECsoil

The PNEC and PNED for terrestrial organisms were calculated in the Annex 1 CAR using the 30-day EC50 for earthworms of 1000 mg/kg soil (corresponding to 4.8 x1010 CFU/kg and 8x106 ITU/kg). An assessment factor of 1000 was applied to give a PNEC of 1 mg/kg soil, which equates to PNEDsoil = 4.8 x107 CFU/kg soil and PNECsoil = 8x103 ITU/kg). It should be noted that in the Annex 1 CAR, a mistake has occurrred and a PNED of 1.0 x107 CFU/kg soil was defined.

#### Summary of PNECs of the active substance *Bacillus thuringiensis subsp. israelensis* Serotype H-14 Strain AM65-52

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Compartment | Species | Endpoint | Safety factor | PNEC - PNED |
| (*Fresh*) Water | Daphnia magna | 0.5 mg/L (1x108 CFU/L; 3.3x103 ITU/L) | 10 | 0.05 mg/L (1x107 CFU/L; 3.3x102 ITU/L) |
| Soil |  | LC50 = 1000 mg/kg dry weight soil (4.8x1010 CFU/kg dw soil; 8x106 ITU/kg dw soil) | 1000 | 1 mg/kg dry weight (4.8x107 CFU/kg dw soil; 8x103 ITU/kg dw soil) |

#### Non compartment specific effect relevant to the food chain

Not applicable.

#### PBT and ED Assessment

Not applicable.

### Effects on environmental organisms for biocidal product

As the active substance *Bacillus thuringiensis* subsp. *israelensis* Serotype H-14 Strain BMP144 is technically equivalent to the approbated active substance *Bacillus thuringiensis* subsp. *israelensis* Serotype H-14 Strain AM65-52, the summary of information about the active substance *Bacillus thuringiensis* subsp. *israelensis* Serotype H-14 Strain BMP144 is carried out with the data from the CAR of *Bacillus thuringiensis* subsp. *israelensis* Serotype H-14 Strain AM65-52 [general CAS N° for Bt’s 68038-71-1] supplied by the applicant Sumitomo Chemical Agr. Europe SAS. (Assessment Report According to Directive 98/8/EC, Active substance in Biocidal Products, *Bacillus thuringiensis* subsp. *israelensis* Serotype H-14 Strain AM65-52, Product Type 18 ([Insecticides](http://en.wikipedia.org/wiki/Insecticide), [acaricides](http://en.wikipedia.org/wiki/Acaricide) and products to control other [arthropods](http://en.wikipedia.org/wiki/Arthropods)), RMS Italy, February 2010.

Considering the definition of a substance of concern set in Guidance on the Biocial Products Regulation (vol IV B+C)[[7]](#footnote-7)[1], AQUABAC DF 3000 does not contain any substance of concern for the environment.

No new studies were conducted with AQUABAC DF 3000.

#### Aquatic compartment (including water, sediment and STP)

##### Aquatic organisms

Refer to section 2.6.2.1.1.

##### Sediment dwelling organisms

Refer to section 2.6.2.1.2.

##### STP micro-organisms

Refer to section 2.6.2.1.3.

#### Atmosphere

Refer to section 2.6.2.2.

#### Terrestrial compartment

Refer to section 2.6.2.3.

#### Non compartment specific effect relevant to the food chain

Refer to section 2.6.2.4.

#### Summary of PNECs

Refer to section 2.6.2.5.

### Environmental exposure assessment

#### Assessment of exposure to the environment

AQUABAC DF 3000 is a solid product (43% w/w of a.s. Bti-BMP144; 6.26E+10 CFU.g-1), with a potency of 3000 ITU.mg-1.The intended uses are aerial and ground applications in the following lentic water system: Flood water, roadside ditches, irrigation ditches, rice fields, pastures, woodland pools, snowmelt pools, standing ponds, standing pools, standing water containing mosquito larvae in fields growing crops such as alfalfa, almonds, asparagus, corn, cotton, dates, grapes, peaches and walnuts, tidal water, salt marshes, catch basins, and storm water retention areas, sewage lagoons, animal waste lagoons.

The application rate is 0.125 to 1 kg.ha-1 of product AQUABAC DF 3000. The maximum number of application per annum was not provided by the applicant. The interval between 2 applications is 7 to 14 days, reducing to 5 days in cases of heavy insect populations.

Two intended uses were considered for the environmental risk assessment of the AQUABAC XT:

1. Ground application of AQUABAC DF 3000 in lentic water systems, considering the approach applied in the Annex 1 CAR, and taking account the specificity of the AQUABAC XT described above.
2. Aerial application of AQUABAC DF 3000 in lentic water systems. Aerial application was not been assessed in the Annex 1 CAR.

#### Intended use 1: Ground application of AQUABAC DF 3000 in lentic water systems

##### Aquatic compartment (surface water, sediment, STP)

**Surface water and sediment**

AQUABAC DF 3000 is directly applied to surface water with at least 5 days between each application. As the maximum number of application per annum was not provided by the applicant, the environmental exposure assessment was performed by considering the maximum number of application used in the CAR (*i.e.* 8 applications per annum). Calculations have been performed for the lowest intended dose (0.125 kg/ha; 7.83E+12 CFU/ha; 3.75E+08 ITU/ha), and the highest intended dose (1kg/ha; 6.26E+13 CFU/ha; 3.00E+09 ITU/ha).

The population densities (EEDsw, CFU/L) and biopotency (PECsw, ITU/L) calculations have been carried out with a first step considering a dilution following an application on a water body of 30 cm of depth and dissipation between two applications (DT50 = 50 days for spores and DT50 = 14 days for toxin, degradation constant = e(-ln(2)/DT50\*5).

In a second step, adsorption of the spores and the toxin have been taken into account considering a Koc of 1000 L/kg and calculated as following equation issued from Annex1 CAR:

EEDsw, t=0 (CFU/L) = rate (CFU/ha) x fL/T / VL (L/ha)

And

PECsw, t=0 (ITU/L) = rate (ITU/ha) x fL/T / VL (L/ha)

With fL/T = 1/(1+ Kadsxx(Ls/LL))

For multiple applications, the populations density (EEDsw, CFU/L) and biopotency (PECsw, ITU/L) in water after n+1 applications were calculated as follows:

EEDSW, init, n+1 = EEDSW, final, n + EEDSW, t=0

and

PECSW, init, n+1 = PECSW, final, n + PECSW, t=0

where:

EEDSW, final, n = EEDSW, init, n xe(-ln(2)/DT50\*5)

and

PECSW, final, n = PECSW, init, n xe(-ln(2)/DT50\*5)

The second step allows to calculating the population densities (EEDsw, CFU/g) and biopotency (PECsw, ITU/g) in sediment as follows. In the Annex I CAR, following calculation are proposed:

EEDSed, init, n+1 = EEDSed, final, n + EEDSed, t=0

and

PECSed, init, n+1 = PECSed, final, n + PECSed, t=0

where:

EEDSed, final, n = EEDSW, final, n xKads/1000

and

PECSed, final, n = PECSW, final, n xKads/1000

and

EEDSed, t=0 = EEDSW, t=0 xKads/1000

and

PECSed, t=0 = PECSW, t=0 xKads/1000

With the following symbols:

LL (m): Depth of the water pounding on sediments

LS (m): Thickness of the sediments

 (kg L-1): Sediment density

EEDSW, t=0: *Bti* initial population density in water considering dilution and adsorption before any dissipation (CFU/L)

EEDSW, init, n: *Bti* population density in water following n applications (CFU/L)

EEDSW, final, n: *Bti*  population density in water after 5 days following n applications (CFU/L)

PECSW, t=0: *Bti* initial biopotency in water considering dilution and adsorption before any dissipation (ITU/L)

PECSW, init, n: *Bti* biopotency in water following n applications (ITU/L)

PECSW, final, n: *Bti* biopotency in water after 5 days following n applications (ITU/L)

EEDSed, t=0: *Bti*  initial population density in sediments (CFU/g)

EEDSed, final, n: *Bti* population density in sediments after 5 days following n applications (CFU/g)

EEDSed, init, n+1: *Bti* population density in sediments following n+1 applications (CFU/g)

PECSed, t=0: *Bti* initial biopotency in sediments (ITU/g)

PECSed, final, n: *Bti* biopotency in sediments after 5 days following n applications (ITU/g)

PECSed, init, n+1: *Bti* biopotency in sediments following n+1 applications (ITU/g)

Inputs and Annex1 CAR defaults which were used in the two-compartment model are presented in the below

Table 2: Inputs and defaults for step 2 water and sediment calculations

|  |  |  |  |
| --- | --- | --- | --- |
| **Constant** | **symbol (unit)** | **Value** |  |
| Application number | - | 8 | S |
| Interval between applications | (days) | 5 | S |
| Application rate | rate (CFU/ha) | Lowest dose:  7.83E+12  Highest dose:  6.26E+13 | S |
| Application rate | Rate (ITU/ha) | Lowest dose:  3.75E+08  Highest dose:  3.00E+09 | S |
| Water dissipation of spores in water | DT50dis  (days) | 50 | S |
| Water dissipation of toxins in water | DT50dis  (days) | 14 | S |
| Depth of the water pounding on sediments | LL (m) | 0.30 | D |
| Volume of water | VL (L/ha) | 3000000 | D |
| Thickness of the sediments | LS (m) | 0.05 | D |
| Sediment density |  (kg L-1) | 1.5 | D |
| Partition coefficient organic carbon-water | Koc (L/kg) | 1000 | S |
| Adsorption constant, considering 5% of organic carbon in sediment | Kads | 50 | O |
| Fraction of total density or biopotency in water | fL/T (-) | 0.0741 | O |

The predicted *Bti* BMP144 density and biopotency in water and sediment are reported in tables below

Table 3: predicted *Bti* BMP144 density and biopotency in water after ground application of AQUABAC DF 3000 at surface water for each application, by considering degradation in water and adsorption on sediment.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Number of application | Estimated density - EED (CFU/L) | | Biopotency - PEC (ITU/L) | |
| Step 1 | Step 2 | Step 1 | Step 2 |
| **Lowest dose:** | **7.83E+12 CFU/ha** | | **3.75E+08 ITU/ha** | |
| 1 | 2.61E+06 | 1.93E+05 | 1.25E+02 | 9.26E+00 |
| 2 | 5.04E+06 | 3.73E+05 | 2.23E+02 | 1.65E+01 |
| 3 | 7.31E+06 | 5.42E+05 | 2.99E+02 | 2.21E+01 |
| 4 | 9.43E+06 | 6.99E+05 | 3.58E+02 | 2.65E+01 |
| 5 | 1.14E+07 | 8.45E+05 | 4.05E+02 | 3.00E+01 |
| 6 | 1.33E+07 | 9.82E+05 | 4.41E+02 | 3.27E+01 |
| 7 | 1.50E+07 | 1.11E+06 | 4.69E+02 | 3.48E+01 |
| 8 | 1.66E+07 | 1.23E+06 | 4.91E+02 | 3.64E+01 |
| **Highest dose:** | **6.26E+013 CFU/ha** | | **3.00E+09 ITU/ha** | |
| 1 | 2.09E+07 | 1.55E+06 | 1.00E+03 | 7.41E+01 |
| 2 | 4.03E+07 | 2.99E+06 | 1.78E+03 | 1.32E+02 |
| 3 | 5.85E+07 | 4.33E+06 | 2.39E+03 | 1.77E+02 |
| 4 | 7.55E+07 | 5.59E+06 | 2.87E+03 | 2.12E+02 |
| 5 | 9.13E+07 | 6.76E+06 | 3.24E+03 | 2.40E+02 |
| 6 | 1.06E+08 | 7.85E+06 | 3.53E+03 | 2.61E+02 |
| 7 | 1.20E+08 | 8.87E+06 | 3.75E+03 | 2.78E+02 |
| 8 | 1.33E+08 | 9.82E+06 | 3.93E+03 | 2.91E+02 |

Table 4: predicted *Bti* BMP144 density and biopotency in sediment after ground application of AQUABAC XT at surface water for each application, by considering degradation in water and adsorption on sediment.

|  |  |  |
| --- | --- | --- |
| Number of application | Estimated density - EED (CFU/g dwt) | Biopotency - PEC (ITU/g dwt) |
| **Lowest dose:** | **7.83E+12 CFU/ha** | **3.75E+08 ITU/ha** |
| 1 | 9.66E+03 | 4.63E-01 |
| 2 | 1.87E+04 | 8.24E-01 |
| 3 | 2.71E+04 | 1.11E+00 |
| 4 | 3.49E+04 | 1.33E+00 |
| 5 | 4.23E+04 | 1.50E+00 |
| 6 | 4.91E+04 | 1.63E+00 |
| 7 | 5.55E+04 | 1.74E+00 |
| 8 | 6.14E+04 | 1.82E+00 |
| **Highest dose:** | **6.26E+013 CFU/ha** | **3.00E+09 ITU/ha** |
| 1 | 7.73E+04 | 3.70E+00 |
| 2 | 1.49E+05 | 6.60E+00 |
| 3 | 2.17E+05 | 8.85E+00 |
| 4 | 2.79E+05 | 1.06E+01 |
| 5 | 3.38E+05 | 1.20E+01 |
| 6 | 3.93E+05 | 1.31E+01 |
| 7 | 4.44E+05 | 1.39E+01 |
| 8 | 4.91E+05 | 1.46E+01 |

**STP**

Applications in sewage lagoons are intended and exposure from this use is therefore briefly presented. For these uses, it is considered that AQUABAC DF 3000 is applied at the same rate as it is applied to other lentic water system, with a maximum of 1 kg/ha. Hence, the risk assessment for the STP is considered covered by the risk assessment for surface water.

It is likely that these values largely overestimate the density of *Bti* in effluent because of a higher dilution in STP than the worst case considered for surface water. In addition, the quantity of organic carbon present in wastewater will likely be far greater than that considered in the surface water calculation, and consequently it is to be expected that there will be increased binding of *Bti* to organic carbon. Hence, the above calculated effluent densities are to be considered conservative.

##### Atmospheric compartment

The results of numerous surveys indicate that *Bti* can be a naturally occurring microbe present at low levels in the environment. The vegetative cells and insecticidal toxins of *Bti* are quickly degraded and although spores of *Bti* are more resistant they do not multiply substantially. Due to the relative instability of *Bti* in the environment, substantial concentrations of the micro-organism will not be present in air unless sprayed aerially and with repeated treatments for extended time periods. Nonetheless, degradation / inactivation by solar radiation has been shown for spores of *Bti* and in several studies for endotoxins and spores of *Btk*, supporting a low persistence of *Bti* in atmospheric compartment even in the case of an important aerially application. Consequently the micro-organism will not undergo long-range atmospheric transportation. The overall conclusion for atmospheric compartment is that substantial concentrations of the micro-organism will not be present in air.

##### Terrestrial compartment (soil and groundwater)

The EED (CFU/kg) and PEC (ITU/kg) in soil was calculated for 8 applications with an interval of 5 days between applications. In accordance with the CAR, it was assumed that AQUABAC DF 3000 was applied directly to soil at the lowest intended dose (0.125 kg/ha; 7.83E+12 CFU/ha; 3.75E+08 ITU/ha) and the highest intended dose (1 kg/ha; 6.26E+13 CFU/ha; 3.00E+09 ITU/ha), without taking any drift factor into account. In addition, as a step 2, the exposure to soil was refined considering a drift value.

Application in STP could lead to indirect exposure of soil trough spreading of sludge. *Bti* is assumed to not actively compete with other microorganisms (please see 2.8.1.2). At present, such exposure in considered to be covered by the assessment carried out for the direct application on surface water and resulting drift to the soil.

The exposure to soil was calculated and refined from the application rates to water (0.125 and 1kg/ha) and using an appropriate drift factor from Rautmann et al. (1999)[[8]](#footnote-8). As these drift factors are issued from pesticides model exposure, we assumed that the product is applied as early application to fruit crops as a worst-case scenario for drift. This is because application to early fruit crops uses airblast application which directs sprays upwards to the crop canopy. In this way, high levels of drift are possible as the spray becomes airborne. Due to the fact that this assessment considers 8 applications of the product, the 67th percentile drift values outlined in the guidance were used. The RMS chose the highest drift value of 22.24%, proposed for a distance of 3 m as a worst case. It should be noted that 3 m of distance could still appears as to high in the case of application in small containers for instance, however in this case, drift from application on fruit crop could really be considered as a worst case.

First order dissipation rates between applications were assumed for both the spores (120 days) and the toxin (5.2 days). The other assumptions used for the calculation of the EED and PEC are :

* incorporation into the top 5 cm layer over 1 ha (e.g. 10000 m2)
* soil density of 1.5 g/ cm³
* no adsorption
* plant interception: 0 %.

EED and PEC are calculated as follows:

EEDsoil, t=0 (CFU/kg) = rate (CFU/ha) x drift (%) / 10000 (m2) / 0.05 (m) /1500 (kg/m3)

And

PECsoil, t=0 (ITU/kg) = rate (ITU/ha) x drift (%) / 10000 (m2) / 0.05 (m) /1500 (kg/m3)

For multiple applications, the populations density (EEDsoil, CFU/kg) and biopotency (PECsoil, ITU/kg) in soil after n+1 applications were calculated as follows:

EEDsoil, init, n+1 = EEDsoil, final, n + EEDsoil, t=0

and

PECsoil, init, n+1 = PECsoil, final, n + PECsoil, t=0

where:

EEDsoil, final, n = EEDsoil, init, n xe(-ln(2)/DT50\*5)

and

PECsoil, final, n = PECsoil, init, n xe(-ln(2)/DT50\*5)

With the following symbols:

EEDsoil, t=0: *Bti* initial population density in soil before any dissipation (CFU/kg)

EEDsoil, init, n: *Bti* population density in soil following n applications (CFU/kg)

EEDsoil, final, n: *Bti* population density in soil after5 days following n applications (CFU/kg)

PECsoil, t=0: *Bti* initial biopotency in soil before any dissipation (ITU/kg)

PECsoil, init, n: *Bti* biopotency in soil following n applications (ITU/kg)

PECsoil, final, n: *Bti* biopotency in soil after 5 days following n applications (ITU/kg)

Results are reported in the table below:

Table 4: predicted *Bti* BMP144 density and biopotency in soil after ground application of AQUABAC DF 3000 at surface water

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Number of application | Estimated density - EED (CFU/kg) | | Biopotency - PEC (ITU/kg) | |
| Step 1 (drift: 100%) | Step 2 (drift: 22.24%) | Step 1 (drift: 100%) | Step 2 (drift: 22.24%) |
| **Lowest dose:** | **7.83E+12 CFU/ha** | | **3.75E+08 ITU/ha** | |
| 1 | 1.04E+07 | 2.32E+06 | 5.00E+02 | 1.11E+02 |
| 2 | 2.06E+07 | 4.57E+06 | 7.57E+02 | 1.68E+02 |
| 3 | 3.04E+07 | 6.76E+06 | 8.89E+02 | 1.98E+02 |
| 4 | 4.00E+07 | 8.89E+06 | 9.56E+02 | 2.13E+02 |
| 5 | 4.93E+07 | 1.10E+07 | 9.91E+02 | 2.20E+02 |
| 6 | 5.83E+07 | 1.30E+07 | 1.01E+03 | 2.24E+02 |
| 7 | 6.71E+07 | 1.49E+07 | 1.02E+03 | 2.26E+02 |
| 8 | 7.56E+07 | 1.68E+07 | 1.02E+03 | 2.27E+02 |
| **Highest dose:** | **6.26E+013 CFU/ha** | | **3.00E+09 ITU/ha** | |
| 1 | 8.35E+07 | 1.86E+07 | 4.00E+03 | 8.90E+02 |
| 2 | 1.65E+08 | 3.66E+07 | 6.05E+03 | 1.35E+03 |
| 3 | 2.43E+08 | 5.41E+07 | 7.11E+03 | 1.58E+03 |
| 4 | 3.20E+08 | 7.11E+07 | 7.65E+03 | 1.70E+03 |
| 5 | 3.94E+08 | 8.77E+07 | 7.93E+03 | 1.76E+03 |
| 6 | 4.66E+08 | 1.04E+08 | 8.07E+03 | 1.80E+03 |
| 7 | 5.37E+08 | 1.19E+08 | 8.14E+03 | 1.81E+03 |
| 8 | 6.05E+08 | 1.35E+08 | 8.18E+03 | 1.82E+03 |

**Groundwater**

Bacillus thuringiensis cells applied to field soils under natural conditions do not move appreciably through the soil profile (see 2.8.1.2). The lack of mobility is attributed to adsorption onto clay minerals and silica. Bacillus thuringiensis parasporal crystal toxins are also rapidly bound to clay particles and will be similarly non-mobile in soil. Substantial concentrations of Bti Strain BMP144 will not be present in groundwater.

##### Non-compartmental-specific exposure relevant to the food chain (secondary poisoning)

The two potential routes for secondary exposure to *Bti* are insect predators ingesting affected larvae or spores being ingested from dead organic matter. However, given the specificity of the mode of action of *Bti* the majority of insect predators of mosquitoes and black fly are not susceptible to *Bti*, the main exception to this are predatory Nematocera. Studies have been reported where various predators were fed a mixture of *Bti* treated or untreated insects with no effects (Lacey and Merritt, 2003). In a study in which grass shrimp (*Palaemonetes vulgaris*) (Christensen, 1990) were exposed to *Bti* via the test media and treated food the shrimp were thought to have ingested and then passed *Bti* without any ill effects. It is considered that the risk of secondary poisoning and toxic effects on organisms at higher trophic levels is unlikely.

#### Intended use 2: Aerial application of AQUABAC DF 3000 in lentic water systems

##### Aquatic compartment (surface water, sediment, STP)

It is assumed that exposure of the aquatic compartment in the case of aerial application is similar or lower than the exposure resulting from ground application. Indeed, higher drift value could be expected for aerial application, leading to lower predicted concentration in aquatic compartment than for the ground application. Therefore, exposure assessment from the aerial application is considered as covered by the assessment carried out for the ground assessment.

##### Atmospheric compartment

Same assumptions than for ground application occur. See 2.6.4.2.2.

##### Terrestrial compartment (soil and groundwater)

For the aerial application, as a step1 no drift value is considered as a worst case. Hence 100% of application of AQUABAC DF 3000 is therefore considered. Additionally, the exposure to soil was calculated using a drift factor according to FOCUS 2011[[9]](#footnote-9). In this document, a drift of 27.3% is considered at 5 m for rice field. Other parameters and calculation are the same as for ground application (see 2.8.4.2.3 for more details). Predicted density and biopotency are reported in the table below.

Table 5: predicted *Bti* BMP144 density and biopotency in soil after aerial application of AQUABAC DF 3000 in soil

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Number of application | Estimated density - EED (CFU/kg dwt) | | Biopotency - PEC (ITU/kg dwt) | |
| Step 1 (drift: 100%) | Step 2 (drift: 27.3%) | Step 1 (drift: 100%) | Step 2 (drift: 27.3%) |
| **Lowest dose:** | **7.83E+12 CFU/ha** | | **3.75E+08 ITU/ha** | |
| 1 | 1.04E+07 | 2.85E+06 | 5.00E+02 | 1.37E+02 |
| 2 | 2.06E+07 | 5.62E+06 | 7.57E+02 | 2.07E+02 |
| 3 | 3.04E+07 | 8.30E+06 | 8.89E+02 | 2.43E+02 |
| 4 | 4.00E+07 | 1.09E+07 | 9.56E+02 | 2.61E+02 |
| 5 | 4.93E+07 | 1.35E+07 | 9.91E+02 | 2.71E+02 |
| 6 | 5.83E+07 | 1.59E+07 | 1.01E+03 | 2.75E+02 |
| 7 | 6.71E+07 | 1.83E+07 | 1.02E+03 | 2.78E+02 |
| 8 | 7.56E+07 | 2.06E+07 | 1.02E+03 | 2.79E+02 |
| **Highest dose:** | **6.26E+013 CFU/ha** | | **3.00E+09 ITU/ha** | |
| 1 | 8.35E+07 | 2.28E+07 | 4.00E+03 | 1.09E+03 |
| 2 | 1.65E+08 | 4.49E+07 | 6.05E+03 | 1.65E+03 |
| 3 | 2.43E+08 | 6.64E+07 | 7.11E+03 | 1.94E+03 |
| 4 | 3.20E+08 | 8.73E+07 | 7.65E+03 | 2.09E+03 |
| 5 | 3.94E+08 | 1.08E+08 | 7.93E+03 | 2.16E+03 |
| 6 | 4.66E+08 | 1.27E+08 | 8.07E+03 | 2.20E+03 |
| 7 | 5.37E+08 | 1.47E+08 | 8.14E+03 | 2.22E+03 |
| 8 | 6.05E+08 | 1.65E+08 | 8.18E+03 | 2.23E+03 |

**Groundwater**

Same assumptions than for ground application occur. See 2.6.4.2.3

##### Non-compartmental-specific exposure relevant to the food chain (secondary poisoning)

Same assumptions than for ground application occur. See 2.6.4.2.4

### Risk characterisation for the environment

In the BTI strain AM695-52 PT18 Assessment Report, the risk assessment has been carried out with CFU. Besides in the Assessment Report, predicted environmental concentration (PEC) and several ecotoxicity endpoints have been expressed as ITU, as a measure of biopotency. Biopotency can be considered as an indirect way to assess the risk of the toxins, which is at present not been assessed. Therefore, a risk assessment based on ITU is reported below.

#### Intended use 1: Ground application of AQUABAC DF 3000 in lentic water systems

##### Aquatic compartment (including water, sediment and STP)

Table 6: PEC/PNEC ratios for different exposure situations concerning the surface water, considering ground application of AQUABAC DF 3000 in lentic water system

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Number of application | Estimated density | | | | | | | | Biopotency | | | | | | | |
| Step 1 | | | | | | Step 2 | | Step 1 | | | Step 2 | | | | |
| EED (CFU/L) | EED/PNED | | | | | EED (CFU/L) | EED/  PNED | PEC (ITU/L) | PEC/  PNEC | | PEC (ITU/L) | | | PEC/PNEC | |
| **Lowest dose:** | **7.83E+12 CFU/ha** | | | | | | | | **3.75E+08 ITU/ha** | | | | | | | |
| 1 | 2.61E+06 | | | 0.26 | 1.93E+05 | | | 1.93E-02 | 1.25E+02 | | 0.38 | 9.26E+00 | | 2.81E-02 | | |
| 2 | 5.04E+06 | | | 0.50 | 3.73E+05 | | | 3.73E-02 | 2.23E+02 | | 0.67 | 1.65E+01 | | 5.00E-02 | | |
| 3 | 7.31E+06 | | | 0.73 | 5.42E+05 | | | 5.42E-02 | 2.99E+02 | | 0.90 | 2.21E+01 | | 6.71E-02 | | |
| 4 | 9.43E+06 | | | 0.94 | 6.99E+05 | | | 6.99E-02 | 3.58E+02 | | **1.09** | 2.65E+01 | | 8.04E-02 | | |
| 5 | 1.14E+07 | | | **1.14** | 8.45E+05 | | | 8.45E-02 | 4.05E+02 | | **1.23** | 3.00E+01 | | 9.08E-02 | | |
| 6 | 1.33E+07 | | | **1.33** | 9.82E+05 | | | 9.82E-02 | 4.41E+02 | | **1.34** | 3.27E+01 | | 9.90E-02 | | |
| 7 | 1.50E+07 | | | **1.50** | 1.11E+06 | | | 0.11 | 4.69E+02 | | **1.42** | 3.48E+01 | | 0.11 | | |
| 8 | 1.66E+07 | | | **1.66** | 1.23E+06 | | | 0.12 | 4.91E+02 | | **1.49** | 3.64E+01 | | 0.11 | | |
| **Highest dose:** | **6.26E+013 CFU/ha** | | | | | | | | **3.00E+09 ITU/ha** | | | | | | | |
| 1 | 2.09E+07 | | **2.09** | | | 1.55E+06 | | 0.155 | 1.00E+03 | | **3.03** | | 7.41E+01 | | | 0.22 |
| 2 | 4.03E+07 | | **4.03** | | | 2.99E+06 | | 0.30 | 1.78E+03 | | **5.40** | | 1.32E+02 | | | 0.40 |
| 3 | 5.85E+07 | | **5.85** | | | 4.33E+06 | | 0.43 | 2.39E+03 | | **7.24** | | 1.77E+02 | | | 0.54 |
| 4 | 7.55E+07 | | **7.55** | | | 5.59E+06 | | 0.56 | 2.87E+03 | | **8.69** | | 2.12E+02 | | | 0.64 |
| 5 | 9.13E+07 | | **9.13** | | | 6.76E+06 | | 0.68 | 3.24E+03 | | **9.81** | | 2.40E+02 | | | 0.73 |
| 6 | 1.06E+08 | | **10.6** | | | 7.85E+06 | | 0.785 | 3.53E+03 | | **10.7** | | 2.61E+02 | | | 0.79 |
| 7 | 1.20E+08 | | **12.0** | | | 8.87E+06 | | 0.89 | 3.75E+03 | | **11.4** | | 2.78E+02 | | | 0.84 |
| 8 | 1.33E+08 | | **13.3** | | | 9.82E+06 | | 0.98 | 3.93E+03 | | **11.9** | | 2.91E+02 | | | 0.88 |

Considering degradation in water and adsorption on sediment (*i.e.* step 2) for the lowest intended dose:

* According to the predicted density, the risk is acceptable after 8 applications every 5 days;
* According to the estimated biopotency, the risk is acceptable after 8 applications every 5 days.

Considering degradation in water and adsorption on sediment (*i.e.* step 2) for the highest intended dose:

* According to the predicted density, the risk is acceptable after 8 applications every 5 days;
* According to the estimated biopotency, the risk is acceptable after 8 applications every 5 days.

RMS considers that biopotency is a good parameter to assess the environmental risk of the toxin contained in Bti, as the biocidal activity of Bti is due to toxin, and the toxin quantity (expressed in ITU) is not directly related to bacteria density (expressed in CFU). However for consistency with the approach proposed by the CAR, the conclusion was based on the density results (CFU).Based on density result the risk is considered acceptable for the surface water after 8 applications every 5 days when the lowest and the highest intended dose are applied.

According to the latest version of the Guidance on active micro-organisms and biocidal products (paragraph IX of the section 6.1.1.1.1)***[[10]](#footnote-10)*** biopotency should be considered as the most relevant unit when the mode of action of the active microorganisms is based on toxins, which the case of Bti. Moreover, also according to this latest version of the Guidance on active micro-organisms and biocidal products, as there is no guidance to determine which assessment factor should be applied to derive the toxicity endpoints for microorganisms, PNEC should not be derived in this case and a semi quantitative risk assessment should be preferred. Therefore, the risk is considered acceptable for both application doses.

For the sediment, predicted density and biopotency have been calculated, however as no PNED or PNEC has been derived, a complete risk assessment cannot be achieved for this compartment. Adsorption on spore on sediment could reduce significantly their availability and toxicity for sediment dwellings organisms. Besides, contradictory results with some predator of targeted organisms are reported in the litterature. Therefore, effects arising from long term and large scale use of the product on natural biological diversity should be assessed.

##### Atmospheric compartment

See 2.6.4.2.2.

##### Terrestrial compartment (including soil and groundwater)

Table 7: PEC/PNEC ratios for different exposure situations concerning the soil after ground application of AQUABAC DF 3000 at soil

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Number of application | Estimated density | | | | | | Biopotency | | | | | |
| Step 1 (drift: 100%) | | | Step 2 (drift: 22.24%) | | | Step 1 (drift: 100%) | | | | Step 2 (drift: 22.24%) | |
| EED (CFU/kg dwt) | EED/PNED | | EED (CFU/kg dwt) | EED/PNED | | PEC (ITU/kg dwt) | | PEC/PNEC | | PEC (ITU/kg dwt) | PEC/PNEC |
| **Lowest dose:** | **7.83E+12 CFU/ha** | | | | | | | **3.75E+08 ITU/ha** | | | | |
| 1 | 1.04E+07 | 0.22 | | 2.32E+06 | | 4.83E-02 | | 5.00E+02 | 6.25E-02 | 1.11E+02 | | 1.39E-02 |
| 2 | 2.06E+07 | 0.43 | | 4.57E+06 | | 9.53E-02 | | 7.57E+02 | 9.46E-02 | 1.68E+02 | | 2.10E-02 |
| 3 | 3.04E+07 | 0.63 | | 6.76E+06 | | 0.14 | | 8.89E+02 | 0.11 | 1.98E+02 | | 2.47E-02 |
| 4 | 4.00E+07 | 0.83 | | 8.89E+06 | | 0.18 | | 9.56E+02 | 0.12 | 2.13E+02 | | 2.66E-02 |
| 5 | 4.93E+07 | **1.03** | | 1.10E+07 | | 0.23 | | 9.91E+02 | 0.12 | 2.20E+02 | | 2.76E-02 |
| 6 | 5.83E+07 | **1.21** | | 1.30E+07 | | 0.27 | | 1.01E+03 | 0.13 | 2.24E+02 | | 2.80E-02 |
| 7 | 6.71E+07 | **1.40** | | 1.49E+07 | | 0.31 | | 1.02E+03 | 0.13 | 2.26E+02 | | 2.83E-02 |
| 8 | 7.56E+07 | **1.58** | | 1.68E+07 | | 0.35 | | 1.02E+03 | 0.13 | 2.27E+02 | | 2.84E-02 |
| **Highest dose:** | **6.26E+013 CFU/ha** | | | | | | | **3.00E+09 ITU/ha** | | | | |
| 1 | 8.35E+07 | | **1.74** | 1.86E+07 | 0.39 | | | 4.00E+03 | 0.50 | 8.90E+02 | | 0.11 |
| 2 | 1.65E+08 | | **3.43** | 3.66E+07 | 0.76 | | | 6.05E+03 | 0.76 | 1.35E+03 | | 0.17 |
| 3 | 2.43E+08 | | **5.07** | 5.41E+07 | **1.13** | | | 7.11E+03 | 0.89 | 1.58E+03 | | 0.20 |
| 4 | 3.20E+08 | | **6.66** | 7.11E+07 | **1.48** | | | 7.65E+03 | 0.96 | 1.70E+03 | | 0.21 |
| 5 | 3.94E+08 | | **8.21** | 8.77E+07 | **1.83** | | | 7.93E+03 | 0.99 | 1.76E+03 | | 0.22 |
| 6 | 4.66E+08 | | **9.72** | 1.04E+08 | **2.16** | | | 8.07E+03 | **1.01** | 1.80E+03 | | 0.22 |
| 7 | 5.37E+08 | | **11.2** | 1.19E+08 | **2.49** | | | 8.14E+03 | **1.02** | 1.81E+03 | | 0.23 |
| 8 | 6.05E+08 | | **12.6** | 1.35E+08 | **2.80** | | | 8.18E+03 | **1.02** | 1.82E+03 | | 0.23 |

Considering drift of 22.24% (*i.e.* step 2), ground application of the lowest intended dose of AQUABAC DF 3000 lead to acceptable risk for the soil after eight applications, by considering density and biopotency results.

Considering drift of 22.24% (*i.e.* step 2), ground application of the highest intended dose of AQUABAC DF 3000 lead to:

* acceptable risk for the soil after a maximum of 2 applications, by considering density result;
* Acceptable risk for the soil, after eight applications, by considering biopotency result.

RMS considers that biopotency is a good parameter to assess the environmental risk of the toxin contained in Bti, as the biocidal activity of Bti is due to toxin, and the toxin quantity (expressed in ITU) is not directly related to bacteria density (expressed in CFU). However for consistency with the approach proposed by the CAR, the conclusion was based on the density results (CFU).

Based on density result the risk is considered:

* Acceptable for the soil after 8 applications every 5 days when the lowest intended dose is applied;
* Acceptable for the soil after a maximum of 2 applications every 5 days when the highest intended dose is applied;

Nevertheless, according to the latest version of the Guidance on active micro-organisms and biocidal products (paragraph IX of the section 6.1.1.1.1)***[[11]](#footnote-11)*** biopotency should be considered as the most relevant unit when the mode of action of the active microorganisms is based on toxins, which the case of Bti. Moreover, also according to this latest version of the Guidance on active micro-organisms and biocidal products, as there is no guidance to determine which assessment factor should be applied to derive toxicity endpoints for microorganisms, PNEC and PNED should not be derived in this case and a semi quantitative risk assessment should be preferred. It should be kept in mind that the PNED is derived from an acute earthworm study showing no adverse effect at the highest tested concentration. An assessment factor of 1000 is applied to this LC50 to derive the PNEDsoil as no other data on soil micro-organisms and plants are submitted. This PNEDsoil could also be considered as very conservative since literature data shows no effect of Bti toxins on soil micro-organisms. Moreover, Bti is considered as not toxic for plants as its mechanism of toxicity is by ingestion and transformation by digestive enzymes to enable the release of the active protein δ-endotoxins. Additionally, the PNED soil value (4.8 x 107 CFU/kg soil) is in the same order of magnitude that the density of Bacillus thuringiensis that occurs in soil (2x105 to 5x107 CFU/kg soil (P.A.W. Martin, 1991).

Therefore, the risk is considered acceptable for both application doses.

##### Non-compartmental specific effects relevant to the food chain (secondary poisoning)

See 2.4.8.2.4

##### Conclusions for ground application of AQUABAC DF 3000 in lentic water systems

The ground applications of the lowest and the highest intended dose (0.125 kg/ha and 1 kg/ha) with an interval of at least 5 days between two applications lead to acceptable risk for the environment, because of:

* + acceptable for the surface water after 8 applications;
  + acceptable for the soil after 8 applications;

For the sediment, as no PNED or PNEC has been derived, a complete risk assessment cannot be achieved for this compartment which was accepted at the EU level for the inclusion of the substance. Besides, contradictory results with some predator of targeted organisms are reported in the litterature. Therefore, it was considered as the EU level that effects arising from long term and large scale use of the product on natural aquatic biological diversity should be assessed.

As a consequence monitoring of effects of the product use on natural biological diversity of treated aquatic system including food webs is required in the case of long term and large scale use of AQUABAC DF 3000.

In this case, it appears more relevant to assess effect of these applications on natural biological diversity, particularly on species closed to targeted species, as for instance other insects belonging to the dipterous sub-order of Nematocera, and species which are trophically related to targeted species, including terrestrial organisms.

#### Intended use 2: Aerial application of AQUABAC DF 3000 in lentic water systems

##### Aquatic compartment (including water, sediment and STP)

It is assumed that exposure of the aquatic compartment in the case of aerial application is similar or lower than the exposure resulting from ground application. Therefore,referred to 2.6.5.1 for this risk characterisation.

##### Atmospheric compartment

See 2.6.4.3.2.

##### Terrestrial compartment (including soil and groundwater)

Table 8: PEC/PNEC ratios for different exposure situations concerning the soil after aerial application of AQUABAC DF 3000 at soil

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Number of application | Estimated density | | | | | | | | Biopotency | | | | | | | |
| Step 1 (drift: 100%) | | | | Step 2 (drift: 27.3%) | | | | Step 1 (drift: 100%) | | | | | | Step 2 (drift: 27.3%) | |
| EED (CFU/kg dwt) | EED/  PNED | | | EED (CFU/kg dwt) | | EED/  PNED | | PEC (ITU/kg dwt) | | | PEC/  PNEC | | | PEC (ITU/kg dwt) | PEC/  PNEC |
| **Lowest dose:** | **7.83E+12 CFU/ha** | | | | | | | **3.75E+08 ITU/ha** | | | | | | | | |
| 1 | 1.04E+07 | 0.22 | | 2.85E+06 | | 5.93E-02 | | 5.00E+02 | | | 6.25E-02 | | | 1.37E+02 | | 1.71E-02 |
| 2 | 2.06E+07 | 0.43 | | 5.62E+06 | | 0.12 | | 7.57E+02 | | | 9.46E-02 | | | 2.07E+02 | | 2.58E-02 |
| 3 | 3.04E+07 | 0.63 | | 8.30E+06 | | 0.17 | | 8.89E+02 | | | 0.11 | | | 2.43E+02 | | 3.03E-02 |
| 4 | 4.00E+07 | 0.83 | | 1.09E+07 | | 0.23 | | 9.56E+02 | | | 0.12 | | | 2.61E+02 | | 3.26E-02 |
| 5 | 4.93E+07 | **1.03** | | 1.35E+07 | | 0.28 | | 9.91E+02 | | | 0.12 | | | 2.71E+02 | | 3.38E-02 |
| 6 | 5.83E+07 | **1.21** | | 1.59E+07 | | 0.33 | | 1.01E+03 | | | 0.13 | | | 2.75E+02 | | 3.44E-02 |
| 7 | 6.71E+07 | **1.40** | | 1.83E+07 | | 0.38 | | 1.02E+03 | | | 0.13 | | | 2.78E+02 | | 3.47E-02 |
| 8 | 7.56E+07 | **1.58** | | 2.06E+07 | | 0.43 | | 1.02E+03 | | | 0.13 | | | 2.79E+02 | | 3.49E-02 |
| **Highest dose:** | **6.26E+013 CFU/ha** | | | | | | | **3.00E+09 ITU/ha** | | | | | | | | |
| 1 | 8.35E+07 | | **1.74** | 2.28E+07 | | | 0.475 | 4.00E+03 | | 0.50 | | | 1.09E+03 | | | 0.14 |
| 2 | 1.65E+08 | | **3.43** | 4.49E+07 | | | 0.94 | 6.05E+03 | | 0.76 | | | 1.65E+03 | | | 0.21 |
| 3 | 2.43E+08 | | **5.07** | 6.64E+07 | | | **1.38** | 7.11E+03 | | 0.89 | | | 1.94E+03 | | | 0.24 |
| 4 | 3.20E+08 | | **6.66** | 8.73E+07 | | | **1.82** | 7.65E+03 | | 0.96 | | | 2.09E+03 | | | 0.26 |
| 5 | 3.94E+08 | | **8.21** | 1.08E+08 | | | **2.24** | 7.93E+03 | | 0.99 | | | 2.16E+03 | | | 0.27 |
| 6 | 4.66E+08 | | **9.72** | 1.27E+08 | | | **2.65** | 8.07E+03 | | **1.01** | | | 2.20E+03 | | | 0.275 |
| 7 | 5.37E+08 | | **11.2** | 1.47E+08 | | | **3.05** | 8.14E+03 | | **1.02** | | | 2.22E+03 | | | 0.28 |
| 8 | 6.05E+08 | | **12.6** | 1.65E+08 | | | **3.44** | 8.18E+03 | | **1.02** | | | 2.23E+03 | | | 0.28 |

Considering drift of 100% (*i.e.* step 1), aerial application of the lowest intended dose (0.125 kg/ha) of AQUABAC DF 300 lead to:

* Acceptable risk for the soil for less than five applications every five days, by considering density result;
* Acceptable risk for the soil after eight applications, by considering biopotency result.

Considering drift of 100% (*i.e.* step 1), aerial application of the highest intended dose (1kg/ha) of AQUABAC DF 3000 lead to:

* Unacceptable risk for the soil for one application, by considering density result;
* Acceptable risk for the soil for less than six applications, by considering biopotency result.

RMS considers that biopotency is a good parameter to assess the environmental risk of the toxin contained in Bti, as the biocidal activity of Bti is due to toxin, and the toxin quantity (expressed in ITU) is not directly related to bacteria density (expressed in CFU). However for consistency with the approach proposed by the CAR, the conclusion was based on the density results (CFU).

As a consequence, the risk for the terrestrial compartment is considered:

* Acceptable for less than five applications every five days when the lowest intended dose is applied;
* Unacceptable even for one application when the highest intended dose is applied.

Taking into account the aerial drift values proposed in the FOCUS Surface Water Scenarios in the EU Evaluation Process under 91/414/EEC[[12]](#footnote-12)[1], less derivation than 100% can be expected. For instance, for aerial application in rice field, a derivation of 27.3% at 5 m could be used for calculations and would lead to acceptable risk for terrestrial compartment even for 8 aerial applications when the lowest intended dose is applied (according to density results).

However, this derivation should be associated with a non treated area and this practice appears as not relevant in the case of the uses against mosquitoes. Indeed, most of the aerial treated areas are large water body as lake, marshland and rice field, and leaving a non treated surface near the bank could have an important adverse effect on the efficacy of the treatment.

Nevertheless, according to the latest version of the Guidance on active micro-organisms and biocidal products (paragraph IX of the section 6.1.1.1.1)***[[13]](#footnote-13)*** biopotency should be considered as the most relevant unit when the mode of action of the active microorganisms is based on toxins, which the case of Bti. Moreover, also according to this latest version of the Guidance on active micro-organisms and biocidal products, as there is no guidance to determine which assessment factor should be applied to derive toxicity endpoints for microorganisms, PNEC and PNED should not be derived in this case and a semi quantitative risk assessment should be preferred It should be kept in mind that the PNED is derived from an acute earthworm study showing no adverse effect at the highest tested concentration. An assessment factor of 1000 is applied to this LC50 to derive the PNEDsoil as no other data on soil micro-organisms and plants are submitted. This PNEDsoil could also be considered as very conservative since literature data shows no effect of Bti toxins on soil micro-organisms.

Moreover, Bti is considered as not toxic for plants as its mechanism of toxicity is by ingestion and transformation by digestive enzymes to enable the release of the active protein δ-endotoxins. Additionally, the PNED soil value (4.8 x 107 CFU/kg soil) is in the same order of magnitude that the density of Bacillus thuringiensis that occurs in soil (2x105 to 5x107 CFU/kg soil (P.A.W. Martin, 1991).

Therefore, the risk is considered acceptable for both application doses.

##### Non-compartmental specific effects relevant to the food chain (secondary poisoning)

See 2.6.4.3.4

##### Conclusions for aerial application of AQUABAC DF 3000 in lentic water systems

The aerial applications of the lowest and the highest intended dose (0.125 kg/ha and 1 kg/ha) with an interval of at least 5 days between two applications lead to acceptable risk for the environment, because of:

* + acceptable for the surface water after 8 applications;
  + acceptable for the terrestrial compartment for 8 applications;

For the sediment, as no PNED or PNEC has been derived, a complete risk assessment cannot be achieved for this compartment which was accepted at the EU level for the inclusion of the substance. Besides, contradictory results with some predator of targeted organisms are reported in the litterature. Therefore, it was considered as the EU level that effects arising from long term and large scale use of the product on natural aquatic biological diversity should be assessed. As a consequence monitoring of effects of the product use on natural biological diversity of treated aquatic system including food webs is required in the case of long term and large scale use of AQUABAC DF 3000.

In this case, it appears more relevant to assess effect of these applications on natural biological diversity, particularly on species closed to targeted species, as for instance other insects belonging to the dipterous sub-order of Nematocera, and species which are trophically related to targeted species, including terrestrial organisms.

***Risk mitigation measures linked to risk assessment for environment***

* Do not exceed 8 applications with an interval of 5 days between two applications.
* The labeling of the product should provide information to the user about the responsibility to follow any local requirements regarding consultation with relevant authority, before the use of AQUABAC DF 3000 in a natural water habitat.
* When applying AQUABAC DF 3000 to ecosystems of great value for biodiversity, i.e. Natura 2000 or nature reserve, specific permission is required.
* The user shall keep records of all uses, including treated areas and concentrations used, for at least 10 years and upon request provide the information to authorities or research.

Disposal Environment

* Dispose of unused product, its packaging and all other waste in accordance with local regulations.
* Do not discharge unused product on the ground, into water courses, into pipes (sink, toilets…) nor down the drains.

## Measures to protect man, animals and the environment

*See Summary of Product Characteristics (SPC)*

# Appendices

Annex 1: List of studies reviewed

***List of new data submitted in support of the evaluation of the biocidal product***

| **Section No / Reference No** | **Author(s)** | **Year** | **Title. Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published** | **Data Protection Claimed (Yes/No)** | **Owner** | **Essential for the evaluation** | |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | | | | | | **Y** | **No** |
|  | | | | | |
| **Section 1** | | | | | |
| IIIA, 1.4.2/01 | Anonymous | 2013 | 5-Batch analysis of *Bacillus thuringiensis* subspecies *israelensis,* Serotype H-14 *(Bti)*  Strain BMP144  Becker Microbial Products Inc, Parkland, Florida, USA  Non-GLP Unpublished  =>Confidential Appendix | Yes | Becker Microbial Products, Inc. |  |  |
| IIIA, 1.4.2/04 | Centrella B | 2014 | Confirmation of the Microbial Levels Present in a Pesticide Product  Eurofins Microbiology Laboratories, Inc. USA  Non-GLP Unpublished  =>Confidential Appendix | Yes | Becker Microbial Products, Inc. |  |  |
| IIIA, 1.4.2/05 | Anonymous | 2013 | Agar plate control log  Becker Microbial Products, Inc.  Non-GLP Unpublished  =>Confidential Appendix | Yes | Becker Microbial Products, Inc |  |  |
| IIIA, 1.4.2/06a | JSC International | 2014 | Review of Microbial Contamination Methods for Bti (BMP 144)  JSC International  Non-GLP Unpublished  =>Confidential Appendix | Yes | CERA SAS |  |  |
| IIIA, 1.4.2/06b | JSC International | 2014 | Validation results for Microbial Contamination methods conducted on Bti (BMP 144)  JSC International  Non-GLP Unpublished  =>Confidential Appendix | Yes | CERA SAS |  |  |
| IIIA, 4. | Brux, A | 2015 | Determination of physico-chemical properties and storage stability tests for AQUABAC DF 3000, , Biogenus | Yes | CERA SAS |  |  |
|  | CERA | 2016 | Courrier ANSES MEEM - bioactivité min, max, nom 1016-signed.pdf  Bioactivié min, max, nom | Yes | CERA |  |  |
|  | CERA | 2016 | Courier ANSES AQUABAC DF 3000 BI.pdf Aquabac DF3000 Biopotency specification | Yes | CERA |  |  |
|  | Akhurst L.C. | 2016 | AQUABAC DF3000: 5 batch analysis for microbial contaminants, CFU count and specificity, Report: NR49CV, Sponsor: CERA SAS, GLP | Yes | CERA |  |  |
|  | Manka S. | 2013 | Determination of physico-chemical properties and storage stability tests for Aquabac DF 3000  Test facility BioGenius GmbH, Bergisch Gladbach, Germany. | Yes | CERA |  |  |
|  | Werner L. | 2017 | Larvicidal efficacy of a product against mosquito larvae, Aedes aegypti 0 week, 12 months and 24 months data (storage at 20 °C) Product AQUABAC DF 3000,  Study: Mo 5073 | Yes | CERA |  |  |
| **Section 2** | | | | | |  |  |
| No study reports submitted | | | | | | | |
| **Section 3** | | | | | |  |  |
| No study reports submitted | | | | | |  |  |
| **Section 4** | | | | | | | |
| IIIA, 4.1/01 | Anonymous | 2005 | BMP 144 Purity Plating  Becker Microbial Products, Inc, Parkland, Florida, USA Not GLP, unpublished | Yes | Becker Microbial Products, Inc. |  |  |
| IIIA, 4.1/02 | Benzon, G.L. | 2001 | Potency Bioassay for *Bacillus thuringiensis* (Bti)  Benzon Research, PA, USA  SOP No. BRP-10-1 Not GLP, unpublished | Yes | Becker Microbial Products, Inc. |  |  |
| IIIA, 4.1/03 | Anonymous | - | Subcutaneous Safety Test in Mice  Becker Microbial Products, Inc, Parkland, Florida, USA Not GLP, unpublished | Yes | Becker Microbial Products, Inc. |  |  |
| IIIA, 4.1/04 | Anonymous | - | Microbiology Methods for Bti  Published Test Methods Not GLP, published | Yes | Public Domain |  |  |
|  | Brux, A | 2015 | Determination of physico-chemical properties and storage stability tests for AQUABAC DF3000, Study: Mo5040, Biogenus | Yes | CERA SAS |  |  |
| **Section 6** | | | | | |  |  |
| IIIB6.10.1 | Tyler, B.M.J. | 1999 | The effectiveness of Aquabac XL (1200ITU/mg) Liquid *B.t*. H-14 Formulation in Reducing Simulium spp. Black Fly Larval Populations under Operational Conditions in Labrador | No | Becker Microbial Products, Inc. |  |  |
| IIIB6.10.2 | Becnel, J.J. | 2005 | Mosquito Efficacy Field Study with Aquabac® DF 3000 | Yes | Becker Microbial Products, Inc. |  |  |
| IIIB6.10.3 | Majori, G.and Ali, A. | 1984 | Laboratory and Field Evaluations of Industrial Formulations of Bacillus thuringiensis serovar. israelensis against some Mosquito Species in Central Italy.  Journal of the Invertebrate Pathology, 43:316-323 | No | Public Domain |  |  |
| IIIB6.10.4 | Gunasekaran, K., Prabakaran, G. and Balaraman, K. | 2002 | Efficacy of a floating sustained release formulation of *Bacillus thuringiensis* spp. *israelensis* in controlling *Culex quinquefasciatus* larvae in polluted water habitats  Acta Tropica, 83:241-347 | No | Public Domain |  |  |
| IIIB6.10.5 | Lee, H.L., Chen, C.D., Mohd Masri, S., Chiang, Y.F., Chooi, K.H and Benjamin, S. | 2008 | Impact of larviciding with a *Bacillus Thuringiensis israelensis* formulation, VECTOBAC WG®, on dengue mosquito vectors in a dengue endemic site in Selangor State, Malaysia  Southeast AsianJ Trop Med Public Health, 39:601-609 | No | Public Domain |  |  |
| IIIB6.10.6 | Al-Sarar, A.S., Al-Shahrani, D., Bayoumi, A.E., Abobakr, Y., and Hussein, H.I. | 2011 | Laboratory and Field Evaluation of Some Chemical and Biological Larvicides against *Culex* spp. (Diptera: Culicidae) Immature Stages  *Int. J. Agric. Biol*., 13: 115–119 | No | Public Domain |  |  |
| IIIB6.10.7 | Rydzanicz, K., DeChant, P. and Becker, N. | 2010 | Field efficiency of granular formulations of *Bacilllus thuringiensis israelensis* – strain AM65-52 against floodwater mosquitoes in Poland and Germany  Journal of the American Mosquito Control Association, 26 (3): 295–301 | No | Public Domain |  |  |
| IIIB6.10.8 | Knepper R.G, Wagner S.A, Walker E.D | 1991 | Aerially applied, liquid Bacillus Thuringiensis Var. israelensis (H-14) for control of spring Aedes mosquitoes in Michigan  Journal of the American Mosquito Control Association, 7 (2), pp 307 – 309 | No | Not specified |  |  |
| IIIB6.10.9 | Knepper R.G, Wagner S.A, Abel, E, Walker E.D | 1994 | Fixed-wing, aerial application of liquid *Bacillus thuringiensis* H-14 (Acrobe) for control of spring Adees mosquitos in Michigan  Journal of the American Mosquito Control Association, 10 (1), pp 42 – 44 | No | Not specified |  |  |
| IIIB6.10.10 | Cornine, F.H. and Deschamps, T.D. | 2012 | CMMCP Aerial Mosquito Larval Control Program – Spring 2012  Central Mass. Mosquito Control Project, 111 Otis Street Northborough, MA 01532 | No | Public Domain |  |  |
| IIIB6.10.11 | EID Méditerranée Laboratory | 2006 | Laboratory evolution of the biological efficiency of the AQUABAC II xt (*Bacillus thuringiensis* ser. *Israelensis*, 1200 UTI/mg, SC) Concerning the *Aedes aegypti* larvae (Diptera: Culicidae) | Yes | S.P.C.I |  |  |
| IIIB6.10.12 | Serrano, B. | 2014 | Laboratory testing of products intended to control mosquitoes – Larvicide effect. T.E.C Laboratory, Anglet, France | Yes | CERA |  |  |
| IIIB6.10.13 | Jeannin, C. | 2015 | Efficacité biologique de la formulation Aquabac® XT à base de Bacillus thuringiensis ser. israelensis par épandage aérien vis-à-vis des larves d’Ochlerotatus (Aedes) caspius (Diptera : Culicidae)  EID Méditerranée - Direction Technique  EID14087 | Yes | CERA SAS |  |  |
| IIIB6.10.14 | Jeannin, C. | 2015 | Efficacité biologique en conditions réelles (parcelles) des formulations Aquabac® XT, Aquabac® 200G et Aquabac® DF3000 à base de Bacillus thuringiensis israelensis vis-à-vis des larves d’Ochlerotatus (Aedes) caspius (Diptera : Culicidae)  EID Méditerranée - Direction Technique  EID14087 | Yes | CERA SAS |  |  |
| **Section 7** | | | | | |  |  |
| No study reports submitted | | | | | | | |
| **Section 8** | | | | | |  |  |
| No study reports submitted | | | | | |  |  |
| **Section 9** | | | | | |  |  |
| No study reports submitted | | | | | |  |  |
| **Section 10** | | | | | |  |  |
| No study reports submitted | | | | | | | |
| **Section 11** | | | | | |  |  |
| No study reports submitted | | | | | |  |  |
| **Section 12** | | | | | |  |  |
| No study reports submitted | | | | | |  |  |
|  | Akhurst L.C. | 2016 | AQUABAC DF3000: 5 batch analysis for microbial contaminants, CFU count and specificity, Report: NR49CV, Sponsor: CERA SAS, GLP | Yes | CERA |  |  |
|  | Manka S. | 2017 | Determination of physico-chemical properties and storage stability tests for Aquabac DF 3000 up to 24 months at 20°C  Test facility BioGenius GmbH, Bergisch Gladbach, Germany.  Study No.: Mo5062  Study director: A. Brux  Start of the study: 15/02/2017  Final report: 15/02/2017 | Yes | CERA |  |  |
|  | Werner L. | 2017 | Larvicidal efficacy of a product against mosquito larvae, Aedes aegypti 0 week, 12 months and 24 months data (storage at 20 °C) Product AQUABAC DF 3000,  Study: Mo 5073 | Yes | CERA |  |  |
|  | Jeannin, C. | 2016 | Efficacité biologique en conditions contrôlées (poubelles) des formulations Aquabac® XT, Aquabac® 200G et Aquabac® DF3000 à base de *Bacillus thuringiensis* ser. *israelensis* vis-à-vis des larves de *Culex quinquefasciatus* (Diptera : Culicidae)  EID Méditerranée - Direction Technique  EID-15RD134-17F | Yes | CERA |  |  |

Annex 2: Analytical methods residues – active substance

**Bacillus thuringiensis israelensis serotype H-14 strain BMP144**

**Matrix, action levels, relevant residue and reference**

|  |  |  |  |
| --- | --- | --- | --- |
| matrix | limit | relevant residue | reference or comment |
| plant products | Not relevant | Not relevant | Not relevant |
| food of animal origin | Not relevant | Not relevant | Not relevant |
| soil | Not relevant | Not relevant | Not relevant |
| drinking water | Not relevant | Not relevant | Not relevant |
| surface water | Not relevant | Not relevant | Not relevant |
| air | Not relevant | Not relevant | Not relevant |
| body fluids / tissues | Not relevant | Not relevant | Not relevant |

Methods suitable for the determination of residues (monitoring methods)

Not relevant, as no MRL were set in in plants, food of animal origin, body fluids, soil, water and air.

Annex 3 : Toxicology and metabolism –active substance

**< Bacillus thuringiensis israelensis AM65-52 >**

**Threshold Limits and other Values for Human Health Risk Assessment**

| **Summary** | | | |
| --- | --- | --- | --- |
|  | Value | Study | SF |
| AEL long-term | Not relevant |  |  |
| AEL medium-term | Not relevant |  |  |
| AEL acute | Not relevant |  |  |
| ADI | Not relevant |  |  |
| ARfD | Not relevant |  |  |
|  | | | |

|  |  |
| --- | --- |
| Inhalative absorption | Not relevant |
| Oral absorption | Not relevant |
| Dermal absorption | Not relevant |

| **Classification** | |
| --- | --- |
| with regard to toxicological data (according to the criteria in Reg. 1272/2008) | Not relevant |

Annex 4 : Toxicology – biocidal product

AQUABAC DF3000

|  |  |
| --- | --- |
| General information | |
| Formulation Type | WG |
| Active substance(s) (incl. content) | Bti BMP 144 (43% w/w technical slurry) |
| Category | PT18 |

| Acute toxicity, irritancy and skin sensitisation of the preparation (Annex IIIB, point 6.1, 6.2, 6.3) | | | | |
| --- | --- | --- | --- | --- |
| Rat LD50 oral (OECD 401) | No data (classification by calculation) |  |  |  |
| Rat LD50 dermal (OECD 402) | No data (classification by calculation) |  |  |  |
| Rat LC50 inhalation (OECD 403) | No data (classification by calculation) |  |  |  |
| Skin irritation (OECD 404) | No data (classification by calculation) |  |  |  |
| Eye irritation (OECD 405) | No data (classification by calculation) H319 |  |  |  |
| Skin sensitisation (OECD 406) | No data (classification by calculation) |  |  |  |

| Additional toxicological information | | | | |
| --- | --- | --- | --- | --- |
| Short-term toxicity studies | None |  |  |  |
| Toxicological data on active substance(s) (not tested with the preparation) | None |  |  |  |
|  |  |  |  |  |
| Toxicological data on non-active substance(s) (not tested with the preparation) | None |  |  |  |
|  |  |  |  |  |
| Further toxicological information | None | | | |

|  |  |
| --- | --- |
| Classification and labelling proposed for the preparation with regard to toxicological properties | |
| Regulation 1272/2008/EC | Eye Irrit Cat.2 H319 Causes serious eye irritation |

Annex 5 : Residue behaviour

**Bacillus thuringiensis israelensis strain BMP144**

Intended Use (critical application)

Active substance(s): *Bacillus thuringiensis* Subsp. *Israelensis,strain BMP 144, 43.0%*

Formulation of biocidal product: Granule containing 3000 ITU/mg of *Bacillus thuringiensis* Subsp. *israelensis*

Place of treatment: outdoor, ground and aerial spray application in water

Control of mosquitoes larvae in water habitats

Applications of 125 g/ha to 500 g/ha for normal habitat and 0.5 kg/ha to 1.0 kg/ha for polluted water,

with intervals of 7 to 14 days and 5 days in case of heavy insect populations, the product can be re-applied as necessary.

The product is to be used to control the number of mosquitoes in water habitats (i.e. flood water, roadside ditches, irrigation ditches, floodwater, rice fields, pastures, woodland pools, snowmelt pools, standing pools, tidal water, salt marshes, catch basins, and storm water retention areas, standing water in fields growing crops (such as alfalfa, almonds, asparagus, corn, cotton, dates, grapes, peaches, and walnuts), sewage lagoons and animal waste lagoons). The intended use descriptions of the *Bti* BMP144 containing biocidal products for which authorisation is sought indicate that these uses are not relevant in terms of residues in food and feed, except for 2 uses (in rice fields and standing water in fields growing crops).

These two intended uses descriptions of the Bti BMP144-containing biocidal products for which authorisation is sought indicate that these uses could lead to an exposure via food consumption.

*Bacillus thuringiensis* has been discussed at EU level from the plant protection products point of view. In an EFSA opinion on *Bacillus cereus* from 2005, it was stated that 103 CFU/g of food could cause food poisoning incidents. In EFSA’s conclusion (EFSA Journal 2012;10(2):2540), a data gap for strain specific residue trials measuring the residues at harvest but also at the point of consumption has been identified. Because standard methods for detection and enumeration of *B. cereus* applied in foodstuff control do not distinguish *B. Cereus sensus stricto* from other Bacillaceae such as *B. thuringiensis* and *Bacillus weihenstephanensis* a general threshold of 105 cfu/g fresh weight is applied in France and some other European countries by food control agencies, irrespective of the food contains pathogenic or non pathogenic *B. cereus* *sensu lato* strain.

*Bti* is a Gram positive, spore forming rod-shaped bacterium that produces a crystalline protein inclusion which is toxic to larvae of some dipteran insects upon ingestion. No studies of residues of *Bacillus thuringiensis subst israelensis* after aerial application with the formulation AQUABAC DF 3000 have been submitted. However, open literature is available. From this literature data it can be shown that both the components spores and crystal proteins are rapidly degraded under UV exposure. Therefore as Bt products are applied to water habitats that are exposed to the sun, the degradation of both viable and non viable residues occurs rapidly and levels of Bt strains introduced by applications decrease rapidly. It was also observed that no multiplication occurs on leaves.

AQUABAC DF 3000 will only be used in rice fields when water is present therefore not at harvest when no water is present. Moreover, rice grains are covered by a husk that is removed prior to consumption. RMS is therefore of the opinion that even if the product applied aerially in water irrigating rice crops can reach food crops located nearby, risk of oral exposure to residues after application via residues of *Bti* on food crops is considered to be negligible. Therefore, the use of AQUABAC DF 3000 in water irrigating rice, with a pre harvest interval of 1 month is acceptable.

However, no data or justification has been given for aerial or ground spray application in standing water in field growing crops (such as alfalfa, almonds, asparagus, corn, cotton, dates, grapes, peaches, and walnuts). As indirect exposure via food cannot be excluded in those cases and without further information, AQUABAC DF 3000 should not be applied in standing water when edible parts of plants are present.

Annex 6: Efficacy of the active substance from its use in the biocidal product

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| --- | --- | --- | --- | --- | --- |
| Test substance | Test organism(s) | Test system / concentrations applied/ exposure time | Test results: effects, mode of action, resistance | Reference | RI |
| AQUABAC DF 3000  (43% *Bacillus thuringiensis*, subsp. *israelensis* (Bt H-14), potency min 3000 ITU/mg) | *Ochlerotatus* (=*Aedes) taeniorhynchus* and *Cx. Quinquefasciatus* | Outdoor potholes 6’x 8’ external dimensions (4’ x 5/ internal dimensions) with sloping sides and a capacity of 60 US gallons were washed and rinsed at least four days prior to flooding using Clorox®. The potholes were filled with water and hog chow added for food (4 gm of hog chow for *Ochlerotatus* (=*Aedes) taeniorhynchus* and 10 gm for *Cx. quinquefasciatus).*  Five hundred late 3rd to early 4th instar larvae of two mosquito species (*Ochlerotatus* (=*Aedes) taeniorhynchus* and *Cx. quinquefasciatus)* were added to each pothole. Two potholes were treated with an equivalent label rate of 100 mg of the AQUABAC® DF 3000, diluted in 1 liter of water and applied with a sprinkling can. The following morning as many larvae as possible were recovered in 25 dips, and the number of live and dead larvae recorded. | The potholes treated with AQUABAC® DF 3000 had 100% mortality and the untreated control potholes had less than 2% (1.6 % mortality for mortality *Oc.taeniorhynchus* and 0.3% mortality for *Cx. quinquefasciatus.*  The recovery rate of the larvae from potholes treated with AQUABAC® DF 3000 was very low:  Only 9.4% (47 out of 500 larvae) for Cx. Quinquefasciatus  And 23% (115 out of 500) for Oc. *Taeniorhynchus.*  So the results of this study are not relevant. | Becnel, J.J. (2005) | 3 |
| AQUABAC XT  (8% *Bacillus thuringiensis*, subsp. *israelensis* (Bt H-14), potency min 1200 ITU/mg)  AQUABAC DF 3000  (43% Bacillus thuringiensis, subsp. israelensis (Bt H-14), potency min 3000 ITU/mg)  AQUABAC 200G  (2.86% Bacillus thuringiensis, subsp. israelensis (Bt H-14), potency min 200 ITU/mg) | *Aedes aegypti*  *Aedes albopictus*  *Culex pipiens*  *Anopheles gambiae*  from laboratory colony breedings | AQUABAC XT, AQUABAC DF3000 and AQUABAC 200G were tested to determine if the product were effective against *Aedes aegypti, Aedes albopictus, Culex pipiens* and *Anopheles gambiae.* The test products were applied to the surface oftest boxes containing 15 3rd and early 4th larval stage mosquitoes. The mosquito species were tested separately. AQUABAC XT was dosed at an application: 25 g of water mixture on 1 m² (6.25 g / 0.25 m² container)  AQUABAC 200G was dosed at an application: 15 g of water mixture on 1 m² (3.75 g / 0.25 m² container)  AQUABAC DF 3000 was dosed at an application: 10 g of water mixture on 1 m² (2.5 g / 0.25 m² container).  Observation recording the mortality of the larvae and the emerging adults after 24 hours, 48 hours and 7 days.  Persistence was investigated using product applied in containers and aged for 15 days. The same procedure as fresh product was used. | Laboratory analysis demonstrated that the dose tested of each product provided 100% reduction in all mosquito larvae strains at observation times of 1,2, 3,4 and 7 days. The efficacy provided 100% reduction for 7 days following use of product treated and aged for 15 days.  The mode of action of Bti on the mosquito is by the ingestion of insecticidal crystal proteins.  The untreated control have proved a fair ratio of emergence of adults along the trial, the trial is validated. | Serrano, B. (2014), T.E.C Laboratory, France | 1 |
| AQUABAC XT  (8% *Bacillus thuringiensis*, subsp. *israelensis* (Bt H-14), potency min 1200 ITU/mg)  AQUABAC DF 3000  (43% Bacillus thuringiensis, subsp. israelensis (Bt H-14), potency min 3000 ITU/mg)  AQUABAC 200G  (2.86% Bacillus thuringiensis, subsp. israelensis (Bt H-14), potency min 200 ITU/mg) | *Ochlerotatus (Aedes) caspius* | Field test  The test took place on 16 and 17 April 2015 on a wetland located in the locality Sainte-Anne in the municipality of St. Laurent-d'Aigouzes (Gard).  The experimental apparatus includes an individual plot of 400 m2 per object. Within each plot are identified twenty sampling points. Just prior to treatment, observed abundances are relatively large, with an average over all plots, 36 larvae per sample. Treatments are performed by ground application in the morning of 04/16/15.  The SC WG and test preparations are diluted with water before use. The application is made using a sprayer Laser® Industry 3610 (13 L) at constant pressure (2 bars), equipped with a suitable hydraulic nozzle air injection (anti-drift - Agricultural approval NTZ). The dilution ratio in the spray mixture is determined before the test to take into account the environmental conditions.  Preparing granulated test (GR) is spread with an atomizer STIHL® SR450.  The 3 plots are manually processed at a speed of 4 km / h. The measured rate of sprayer is 0.72 l / min, that of the atomizer of 0.33 kg / min.  Dose rates were:  AQUABAC® XT: 25.2 L/ha equivalent to 2.9 L of formulated product / ha.  **AQUABAC® DF3000: 24.9 L/ha equivalent to 1.15 kg of formulated product / ha.**  AQUABAC® 200G: 11.8 kg / ha equivalent to 11.8 kg of formulated product / ha.  Environmental conditions (T°C, HR, wind, water T°C, salinity, pH, conductivity, water depth) are measured at T0 and T+24h.  Effectiveness (abundance of mosquito larvae) was evaluated 24h after treatment. | Before treatment, the abundances observed in all plots are average. All the collected larvae are stage L2 (young larvae).  After 24 hrs, the observed abundances are very low with 1 larva by taking an average across all plots. The formulations of AQUABAC® XT, of AQUABAC® DF3000, and of AQUABAC® 200G achieved respectively 96.7%, 98.1%, and 95.4% efficiency in 24 hours. | Jeannin, C. (2015),  EID Méditerranée - Direction Technique | 1 |
| AQUABAC XT  (8% *Bti*, min 1200 ITU/mg)  AQUABAC DF 3000  (43% *Bti*, min 3000 ITU/mg)  AQUABAC 200G  (2.86% *Bti*, min 200 ITU/mg) | *Culex quinquefasciatus*  L2 to L3 larvae | Semi-field test  The test was carried out in 80L LDPE bins. The tested products and controls were replicated 5 times (5 bins). Three days before treatment, each of the 25 bins was filled with 50 L of tap water. A mosquito net was placed on each of the bins to avoid the introduction of insects. The bins were divided into a 5x5 Latin square.  50 L2/L3 larvae were introduced into each bin 1 hour before treatment. 50 larvae are then introduced successively at D3, D7, D10, D14 and D17. At D10, tap water is added to compensate for evaporation. 72 hours after each introduction, Larvae were collected using a dip net and mortality was assessed. The operation was carried out until efficiency <50%.  SC and WG preparations were diluted with water before use. GR preparations were weight before use.  Application rates :  AQUABAC® XT: 35 µL / 0.135 m² => 2.5 L of formulated product / ha.  **AQUABAC® DF3000: 13.5 mg / 0.135 m² => 1kg of formulated product / ha.**  AQUABAC® 200G: 202 mg / 0.135 m² => 15 kg of formulated product / ha. | The test took place from 1st September to 17th November 2016, outdoor, in a courtyard with a sunny exposure.  Environmental conditions (T°C, rainfall, HR, water T°C) are measured during the entire experiment.  At D0: AQUABAC® XT, AQUABAC® DF3000, and AQUABAC® 200G achieved respectively 98%, 99.2%, and 99.6% efficacy.  At D3: AQUABAC® XT, AQUABAC® DF3000, and AQUABAC® 200G achieved respectively 77.6%, 92.8%, and 93.6% efficacy.  Then, mortality was <90% and decreased rapidly until the end of the test. An exception was observed for AQUABAC 200G which showed 94% efficacy at D10 but 84.8% at D7.  Mortality of the control was < 5% during the entire trial. | Jeannin, C. (2016),  EID Méditerranée - Direction Technique | 1 |
| AQUABAC XT  (8% Bti, min 1200 ITU/mg)  AQUABAC DF 3000  (43% Bti, min 3000 ITU/mg) | *Culex quinquefasciatus*  *SLab strain*  *Aedes albopictus*  *SPAM strain*  *L2 to L3 larvae* | Semi-field test  The test took place from 27th to 31th May 2019, outdoor, in a courtyard with a sunny exposure.  The test was carried out in 80L LDPE bins. The tested products and controls were replicated 5 times (5 bins). One day before treatment, each of the 25 bins was filled with 50 L of tap water. A mosquito net was placed on each of the bins to avoid the introduction of insects. The bins were divided into a 5x5 Latin square.  50 L2/L3 larvae were introduced into each bin 1 hour before treatment. 48 and 96 hours after introduction, larvae were collected using a dip net and mortality was assessed.  Environmental conditions (T°C, rainfall, HR, water T°C) were measured during the entire experiment.  SC and WG preparations were diluted with water before use. Application rates :  AQUABAC® XT: 35 µL / 0.135 m² => 2.6 L of formulated product / ha.  **AQUABAC® DF3000: 13.5 mg / 0.135 m² => 1kg of formulated product / ha.**  The test is validated if the average mortality of the controls remains between 0 and 5% and invalidated if the mortality is greater than 20%. When mortality of the control is between 5 and 20%, the mortality percentage is corrected using the Abbott's formula. | At D2: AQUABAC® XT achieved 90.4%, and 100% efficacy respectively against *Cx. quinquefasciatus* and *Ae. albopictus*.  At D4: AQUABAC® XT achieved 98.8% efficacy against Cx. quinquefasciatus.  At D2: AQUABAC® DF3000 achieved 100% against *Ae. albopictus*.  Mortality of the control:  *Cx. Quinquefasciatus*: 1,6% at 48 h and 12 % at 96 h.  *Ae. Albopictus* : 2,4 % at 48 h and 6,4% at 96 h. | Jeannin, C. (2019),  EID Méditerranée - Direction Technique  Report n°EID-18RD13420F | 1 |

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2. Equivalente to the active substance *Bacillus thuringiensis israelensis, serotype H14*, strain AM65-52 included into Annex I of directive 98/8/EC [↑](#footnote-ref-2)
3. CNEV, Utilisation des insecticides et gestion de la résistance, février 2014

   Tetrau, 2012, Devenir du bioinsecticide *Bti* dans l’environnement etimpact sur le développement de résistances chez le moustique [↑](#footnote-ref-3)
4. Assessment Report for the inclusion in annex I of Directive 98/8/EC of the active substance *Bacillus thuringiensis* subsp. *israelensis* Serotype H-14 Strain AM65-52 for Product Type 18 ([Insecticides](http://en.wikipedia.org/wiki/Insecticide), [acaricides](http://en.wikipedia.org/wiki/Acaricide) and products to control other [arthropods](http://en.wikipedia.org/wiki/Arthropods)), RMS Italy, February 2010. [↑](#footnote-ref-4)
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10. Guidance on BPR : Volume V Guidance on active micro-organisms and biocidal products Version 2.1 March 2017 [↑](#footnote-ref-10)
11. Guidance on BPR : Volume V Guidance on active micro-organisms and biocidal products Version 2.1 March 2017 [↑](#footnote-ref-11)
12. [1] FOCUS (2011). "FOCUS Surface Water Scenarios in the EU Evaluation Process under 91/414/EEC". Report of the FOCUS Working Group on Surface Water Scenarios, EC Document Reference SANCO/4802/2001-rev.2. 245 pp.; 2001; updated version 2011. [↑](#footnote-ref-12)
13. Guidance on BPR : Volume V Guidance on active micro-organisms and biocidal products Version 2.1 March 2017 [↑](#footnote-ref-13)