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

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

(submitted by the evaluating Competent Authority)



VECTOBAC WG

Product type 18

Bacillus thuringiensis subsp. Israelensis Sérotype H14 (Bti) souche AM65-52

Case Number in R4BP: BC-FE010761-63

Evaluating Competent Authority: France

Date: June 2015

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Contents

[1 General information about the product application 2](#_Toc422476323)

[1.1 Applicant 4](#_Toc422476324)

[1.2 Person authorised for communication on behalf of the applicant 4](#_Toc422476325)

[1.3 Proposed authorisation holder 4](#_Toc422476326)

[1.4 Information about the product application 5](#_Toc422476327)

[1.5 Information about the biocidal product 5](#_Toc422476328)

[1.5.1 General information 5](#_Toc422476329)

[1.5.2 Information on the intended use(s) 5](#_Toc422476330)

[1.5.3 Information on active substance(s) 7](#_Toc422476331)

[1.5.4 Information on the substance(s) of concern 8](#_Toc422476332)

[1.6 Documentation 9](#_Toc422476333)

[1.6.1 Data submitted in relation to product application 9](#_Toc422476334)

[1.6.2 Access to documentation 10](#_Toc422476335)

[2 Summary of the product assessment 11](#_Toc422476336)

[2.1 Identity related issues…………………………………………………………………………………………11](#_Toc422476337)

[2.2 Classification, labelling and packaging 11](#_Toc422476338)

[2.2.1 Classification of the active substance 11](#_Toc422476339)

[2.2.2 Classification of the biocidal product VECTOBAC WG 11](#_Toc422476340)

[2.2.3 Labelling of the biocidal product 11](#_Toc422476341)

[2.2.4 Packaging of the biocidal product 11](#_Toc422476342)

[2.3 Physico/chemical properties and analytical methods 11](#_Toc422476343)

[2.3.1 Active ingredient 11](#_Toc422476344)

[2.3.2 Biocidal product 15](#_Toc422476345)

[2.3.2.3.1 Methods for microbial active substance 33](#_Toc422476348)

[2.3.2.3.1 Methods for microbial contaminants 36](#_Toc422476349)

[2.4 Risk assessment for Physico-chemical properties and analytical methods 43](#_Toc422476359)

[2.5 Effectiveness against target organisms 46](#_Toc422476365)

[2.5.1 Function 46](#_Toc422476366)

[2.5.2 Organisms to be controlled and products, organisms or objects to be protected 46](#_Toc422476368)

[2.5.3 Effects on target organisms and efficacy 46](#_Toc422476370)

[2.5.4 Mode of action including time delay 49](#_Toc422476385)

[2.5.5 Occurrence of resistance – resistance management / Unacceptable Effect 49](#_Toc422476386)

[2.5.6 Evaluation of the Label Claims 50](#_Toc422476387)

[2.5.7 Summary of efficacy assessment 50](#_Toc422476388)

[2.6 Description of the intended use(s) 50](#_Toc422476391)

[2.7 Risk assessment for human health 51](#_Toc422476393)

[2.7.1 Hazard potential 51](#_Toc422476394)

[2.7.2 Human exposure assessment 55](#_Toc422476401)

[2.7.3 Risk assessment for human health 56](#_Toc422476404)

[2.8 Risk assessment for the environment 59](#_Toc422476411)

[2.8.1 Fate and distribution in the environment of *Bacillus thuringiensis* subsp. *israelensis* Serotype H-14 Strain AM65-52 59](#_Toc422476412)

[2.8.2 Effects on environmental organisms for *Bacillus thuringiensis* subsp. *israelensis* Serotype H-14 Strain AM65-52 60](#_Toc422476414)

[2.8.3 Effects on environmental organisms for biocidal product 68](#_Toc422476422)

[2.8.4 Environmental exposure assessment 69](#_Toc422476428)

[2.8.5 Risk characterisation for the environment 75](#_Toc422476453)

[2.9 Measures to protect man, animals and the environment 79](#_Toc422476455)

[3 Proposal for decision 80](#_Toc422476456)

[4 Appendices 84](#_Toc422476474)

[Annex 0a: Practical use claimed by the applicant for VECTOBAC WG 84](#_Toc422476475)

[Annex 0b: Proposed uses for the authorization of VECTOBAC WG 92](#_Toc422476476)

[Annex 1: Summary of product characteristics 95](#_Toc422476477)

[Annex 2: List of studies reviewed 96](#_Toc422476478)

[Annex 3: Analytical methods residues – active substance 111](#_Toc422476479)

[Annex 4 : Toxicology and metabolism –active substance 112](#_Toc422476484)

[Annex 5 : Toxicology – biocidal product 113](#_Toc422476487)

[\_Toc422476488](#_Toc422476488)[Annex 6 : Safety for professional operators 114](#_Toc422476489)

[Annex 7 : Safety for non-professional operators and the general public 115](#_Toc422476493)

[Annex 8 : Residue behaviour 116](#_Toc422476498)

[Annex 9: Efficacy of the active substance from its use in the biocidal product (\*) 118](#_Toc422476500)

**Note to the reader**

This consolidated PAR for the product authorisation of VECTOBAC WG is based on the PAR of the first authorisation, in which post-authorisation data assessment have been included.

1. **History of the dossier**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Application type** | **refMS** | **Case number in the refMS** | **Decision date** | **Assessment carried out (i.e. first authorisation / amendment /)** |
| NA-APP | FR CA | BC-FE010761-63 | 29/06/2016 | First authorisation |
| NA | FR CA | NA | 2018 | Post-Autorisation data (received on the 16/11/2017) |
| NA-ADC | FR CA | BC-CM033377-38 | 17/10/2017 | Addition of a manufacturing site of the active substance. |
| NA | FR CA | NA | NA | Post-Autorisation data (received on the 21/07/2020; 28/10/2020, 16/12/2020 and 26/03/2021) |

# Conclusion:

# Post authorisation requirement in the first authorisation– 29/06/2016:

The following data were required in post authorisation within 6 months after the first authorisation:

* Maximum biopotency of the product
* The determination of *Salmonella* and of yeast and mould in five batches of the product Vectobac WG using validated methods or international standard methods according to OECD 65 (Oct. 2011)
* The determination of *Staphylococcus aureus* in five batches of the product Vectobac WG using validated method with a limit of detection lower than 10 CFU/g according to OECD 65 (Oct. 2011) (abcence in 1 g)
* Test of the spontaneity of dispersion at the maximaum use concentration (40 %(w/v))
* The detail of composition of the claimed packaging fiber drum

The following data were required in post authorisation within 24 months after the first authorisation:

* The determination of microbial contaminants according to the document OECD 65 (oct. 2011) in the same batch of the product Vectobac WG before and after 24 months at 25 °C
* A study of the persistent foaming before and after 24 months at 25 °C in the same batch of the product according to FAO manual (2010) and according to the GIFAP n° 17 (2009)

# Post-authorisation data – 16/11/2017:

The following data were provided:

* Maximum biopotency of the product (3800 ITU/mg) and is considered **acceptable**
* The determination of the *Salmonella* and *Staphylococcus aureus* in five batches of the product Vectobac WG using a validated method with a limit of detection of 10 CFU/g instead of **“**absent in 25 g” and “absent in 1 g” respectively, the results are acceptable. **Nevertheless,** **the confirmation of absence in 25 g for *Salmonella* and in 1 g for *Staphylococcus aureus* was missing**.
* The detail of the specifications of packaging “Fiber drum” and is considered acceptable.

The following data are still required:

* The determination of the *Salmonella* and *Staphylococcus aureus* in five batches of the product Vectobac WG using a validated method with the criteria of absence in 25 g and absence in 1 g respectively according to the document OECD 65 (oct. 2011), for confirmation.
* The determination of yeast and mould in five batches of the product Vectobac WG using validated methods or international standard methods according to OECD 65 (Oct. 2011).
* The test of the spontaneity of dispersion at the maximaum use concentration (40 %(w/v))
* The determination of microbial contaminants according to the document OECD 65 (oct. 2011) in the same batch of the product Vectobac WG before and after 24 months at 25 °C
* A study of the persistent foaming before and after 24 months at 25 °C in the same batch of the product according to FAO manual (2010) and according to the GIFAP n° 17 (2009)

# Post-authorisation data received – 21/07/2020; 28/10/2020; 16/12/2020:

**The following data were provided and considred acceptable:**

* An acceptable justification for the spontaneity of dispersion at the maximum use concentration (40 %(w/v)), The product should be stired before and during application.

**The following data were not provided but the data gap can be considred acceptable:**

* The confirmation of absence of the *Salmonella* and *Staphylococcus aureus* in five batches of the product Vectobac WG using a validated method with the criteria of absence in 25 g and absence in 1 g respectively according to the document OECD 65 (oct. 2011). Nevertheless, as the claimed uses is non-food uses and as acceptable results in 10 g of five batches of Vectobac WG are available, no more confirmatory data were required.
* The determination of microbial contaminants according to the document OECD 65 (oct. 2011) in the same batch of the product Vectobac WG before and after 24 months at 4 °C. The results can be considered acceptable **but cannot be extrapolated at 25 °C. Considering the absence of the results at 25 °C the mitigation measure should be changed to: “Do not store at temperature above 4 °C” instead of “Do not store at temperature above 25°C”**

**The following data were not provided and remain missing:**

* The determination of yeast and mould in five batches of the product Vectobac WG using validated methods or international standard methods according to OECD 65 (Oct. 2011).
* A study of the persistent foaming before and afer 24 months at 25 °C in the same batch of the product according to FAO manual (2010) and according to the GIFAP n° 17 (2009).

It should be noted that a stability study plan (24 months at 25 °C) for the determination physical and chemical properties on the product VBC-60782 (100% Bti AM 65-52) was provided. **Nevertheless, as the product is different from Vectobac WG, the results of persistent foaming on VBC-60782 could not be used to conclude on Vectobac WG**.

* The determination of microbial contaminants before and after 24 months at 25°C on the product Vectobac WG.
* **Post-authorisation data – March 2021 :**

**The following data were provided and considred acceptable:**

* The determination of yeast and mould in five batches of the product Vectobac WG using validated methods or international standard methods according to OECD 65 (Oct. 2011) was provided and considered acceptable.
* The determination of microbial contaminants before and after 24 months at 4 °C (instead of 25 °C) on the product Vectobac WG. Therefore, the risk mitigation measure “Do not store at temperatures higher than 4°C.” has to be added.

**The following data were not provided and remain missing. These data have to be submitted in the application for renewal of the product authorisation:**

* A study of the persistent foaming before and after 24 months adapted to the recommended storage temperature of the product and according to FAO manual (2010) and according to the GIFAP n° 17 (2009).

It should be noted that a stability study plan (24 months at 25 °C) for the determination physical and chemical properties on the product VBC-60782 (100% Bti AM 65-52) was provided. **Nevertheless, as the product is different from Vectobac WG, the results of persistent foaming on VBC-60782 could not be used to conclude on Vectobac WG.**

# General information about the product application

## Applicant

|  |  |
| --- | --- |
| Company Name: | Sumitomo Chemical Agro Europe SAS |
| Address: | Parc d’Affaires de Crécy, 2 rue Claude Chappe |
| City: | Sain Didier au Mont d’Or |
| Postal Code: | 69771 |
| Country: | France |
| Telephone: | +33478643260 |
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## Person authorised for communication on behalf of the applicant

|  |  |
| --- | --- |
| Name: | 1. LEPLUS Nadine 2. MUNDAY Denise |
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## Proposed authorisation holder

|  |  |
| --- | --- |
| **Company Name:** | Sumitomo Chemical Agro Europe SAS |
| **Address:** | Parc d’Affaires de Crécy, 2 rue Claude Chappe |
| **City:** | Sain Didier au Mont d’Or |
| **Postal Code:** | 69771 |
| **Country:** | France |
| **Telephone:** | +33478643260 |
| **Fax:** | +33478472545 |
| **E-mail address:** | Sylvia.plak@sumitomo-chem.fr |
| **Letter of appointment for the applicant to represent the authorisation holder provided (yes/no):** | NO |

## Information about the product application

|  |  |
| --- | --- |
| Application received: | 11/07/2013 |
| Application reported complete: | 24/09/2013 |
| Type of application: | First product authorisation |
| Further information: |  |

## Information about the biocidal product

### General information

|  |  |
| --- | --- |
| **Trade name:** | VECTOBAC WG |
| **Manufacturer’s development code number(s), if appropriate:** | - |
| **Product type:** | Main Group 3: Pest control.  PT 18: Insecticide |
| **Composition of the product (identity and content of active substance(s) and substances of concern; full composition see confidential annex):** | VECTOBAC WG contains:  *Bacillus thuringiensis* subsp. *israelensis*, strain AM65-52 (37.4% w/w fermentation solids and solubles), formulated as a water dispersible granule with a potency of 3000 ITU/mg. |
| **Formulation type:** | Water dispersible granule |
| **Ready to use product (yes/no):** | Yes |
| **Is the product the very same (identity and content) to another product already authorised under the regime of directive 98/8/EC (yes/no);**  **If yes: authorisation/registration no. and product name:**  **or**  **Has the product the same identity and composition like the product evaluated in connection with the approval for listing of active substance(s) on to Annex I to directive 98/8/EC (yes/no):** | NO |

### Information on the intended use(s)

|  |  |
| --- | --- |
| **Overall use pattern (manner and area of use):** | VECTOBAC’ WG is used for the control of mosquito larvae in water where mosquito breeding occurs. Example areas of use are:  Irrigation ditches, reservoirs, lakes, rivers, flood plains, rice fields, canals, marshland, ponds, catch basins, drainage and roadside ditches, waters in irrigated crops, waste water, sewage effluent/lagoons, septic ditches, animal waste lagoons, natural/manmade containers.  VECTOBAC WG is a selective bacterial larvicide that can be applied to areas that contain fish, other aquatic life and plants. VECTOBAC WG can be applied to areas used by or in contact with humans, pets, horses, livestock, birds, or wildlife. |
| **Target organisms / stages:** | Larval stages L1-early L4 of house mosquitoes such as *Culex spp.* and *Culistea spp.*; *Anopheles spp*.; and floodwater mosquitoes *Aedes spp*. and *Ochlerotatus spp*. |
| **Category of users:** | Professional users |
| **Directions for use including minimum and maximum application rates, application rates per time unit (e.g. number of treatments per day), typical size of application area:** | **Application rate**  VECTOBAC WG may be applied to any water where mosquitoes breed. Dose rates in water which is known to be mosquito larval habitats range from 0.125 - 1.0 kg/ha depending on the population density and water quality. The lowest dose rates provide adequate control of 1st through early 4th instar larvae under most conditions. In cases of a predominance of 4th instar larvae, high population densities, water containing high levels of organic matter, colder temperatures, and/or significant water exchange, higher rates should be used to provide good control of mosquitoes.  In organic rich environment the Bti protein can bind on organic matters and is not available for the larvae to ingest.  **Methode of application:**  VECTOBAC’ WG may be applied using conventional ground or aerial application equipment, with quantities of water sufficient to provide uniform coverage of the target area and according to the type of equipment being used. VECTOBAC WG must be dispersed in water prior to application. VECTOBAC WG suspends readily in water and will stay suspended over normal application periods. Brief recirculation may be necessary if the spray mixture has sat for several hours or longer.  The minimum water volume has been described as 2.5L/ha.  Please note that applications are made by professional users who have years of experience of mosquito control.  The volume of water is more dependent on the use site than the application equipment used. In terrestrial situations where the aquatic environment to which VECTOBAC WG is to be applied is surrounded by dense vegetation, such as reeds, then a high pressure hose may be used to get treatment to the correct cover of the area.  This could take several thousand litres of water without the dose of VECTOBAC WG increasing.  Typical application equipment:   * Portable pump pressure sprayer: from 2,5 to 500 litres per Hectare * Motorized portable blower: from 2,5 to 500 litres per Hectare * Vehicle mounted motorized spray equipment: from 2.5 to 1.000 litres per Hectare.   Aerial application: from 2.5 to 500 litres per Hectare, or when frozen as ice granules using a granule spreader.  **Number and timing of applications:**  The maximum number of applications is 8 with a minimum of 7 days interval between applications. The active ingredient in ‘VECTOBAC’ WG (*Bti* Strain AM65-52) is specific tolarvae of certain *dipteran* insect species. Consequently *‘*VECTOBAC’ WG is a larvicide and the timing of application will depend on the level of larvae infestation and growth stage. The product should be applied during the first to the 4th larval instar, since during the later part of the 4th instar growth stage the larvae are no longer eating and the product will not be effective. |
| **Potential for release into the environment (yes/no):** | Yes |
| **Potential for contamination of food/feedingstuff (yes/no)** | Yes |
| **Proposed Label:** | See the label |
| **Use Restrictions:** | Professional users only |

For full details of the intended uses claimed by the applicant, please see annex 0

### Information on active substance(s)[[1]](#footnote-1)

|  |  |
| --- | --- |
| **Active substance name:** | *Bacillus thuringiensis subsp. Israelensis* serotype H14 strain AM65-52 |
| **CAS No:** | Not applicable |
| **EC No:** | Not applicable |
| **Purity (minimum, g/kg or g/l):** | The technical grade of Bti Strain AM65-52 is a fermentation slurry that contains the bacillus, spores and insecticidal toxins and solid residues from the fermentation. Fermentation residues will include the original components of the fermentation medium, plus metabolic and excretion products from the growing bacteria. The fermentation slurry contains nominally 14% Bti Strain AM65-52, with high and low limits of 20% and 8%, respectively. |
| **Inclusion directive:** | 2011/78/UE (*Bacillus thuringiensis subspecies israelensis (Strain AM65-52)* |
| **Date of inclusion:** | 01/10/2013 |
| **Is the active substance equivalent to the active substance listed in Annex I to 98/8/EC (yes/no):** | Yes |
| **Manufacturer of active substance(s) used in the biocidal product:** |  |
| **Company Name:** | 1. Valent BioSciences Corporation 2. Abbott Laboratories |
| **Address:** | 1. 870 Technology Way 2. Chemical and Agricultural Products Division; 1401 Sheridan Road |
| **City:** | 1. Libertyville, Illinois 2. North Chicago; Illinois |
| **Postal Code:** | 1. 60048 2. 60064 |
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### Information on the substance(s) of concern[[2]](#footnote-2)

Table 1: information on the substance of concern

|  |  |  |  |
| --- | --- | --- | --- |
| **Common name (2.1)** | *Bti* Strain AM65-52 | | |
| **Taxonomic names (2.2)** | **Species:** | | *thuringiensis* |
| **Subspecies:** | | *israelensis* |
| **Serotype:** | | H-14 |
| **Strain:** | | AM65-52 |
| **Genus:** | | *Bacillus* |
| **Family:** | | Bacillaceace |
| **Collection and culture reference number** | SD-1276 American type culture collection | |  |
| **Purity in the technical active substance (fermentation slurry):** | **Minimum** | **Maximum** | **Nominal** |
| 8.0 % | 20 % | 14 % |
| 8789 ITU/mg | 10623 ITU/mg | / |

According to the applicant the nominal bioactivity for the slurry of the fermentation is not usually set, as fermentations can vary and the amount of centrifugation or evaporation is used to ensure proper potencies for the final formulated product.

## Documentation

### Data submitted in relation to product application

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

VECTOBAC WG is the representative product submitted for the annex I of Bti-AMM65-52) inclusion of directive 98/8/CE Physico-chemical properties studies and analytical methods on the biocidal product VECTOBAC WG provided are the same evaluated at EU level in the CAR (Oct. 2010).

**Efficacy data**

**Laboratory tests**

* Laboratory test according to WHO 2005[[3]](#footnote-3) method with the product VECTOBAC WG (37 % w/w Bti AM65-52) on Anopheles sp.
* Laboratory test with the product VECTOBAC WG (37 % w/w Bti AM65-52) according to an internal method on Anopheles gambiae.

**Semi-field tests**

* Semi-field test conducted in Greece with the product VECTOBAC WDG (37 % w/w Bti AM65-52)according to an internal method on Culex pipiens.
* Semi-field test conducted in Spain with the product VECTOBAC WDG (37 % w/w Bti AM65-52) according to an internal method on Aedes albopictus.
* Semi-field test conducted in Spain with the product VECTOBAC WDG (37 % w/w Bti AM65-52) according to an internal method on Aedes albopictus.
* Semi-field test conducted in France with the product VECTOBAC WG (37 % w/w Bti AM65-52) according to an internal method on Culex pipiens.
* Semi-field test conducted in France with the product VECTOBAC WG (37 % w/w Bti AM65-52) according to an internal method on Culex pipiens.
* Semi-field test conducted in Italy with the product VECTOBAC WG (37 % w/w Bti AM65-52) according to an internal method on Culex pipiens.

**Field tests**

* Field test conducted in USA with the product VECTOBAC WDG (37 % w/w Bti AM65-52) according to an internal method on four mosquitoes’s species (Aedes vexans, Culiseta annulata, Ochlerotatus sticticus and Aedes rossicus).
* Field test conducted in Spain with the product VECTOBAC WDG (37 % w/w Bti AM65-52) according to an internal method on Aedes (Ochlerotatus) caspius.
* Field test conducted in Poland with the product VECTOBAC WDG (37 % w/w Bti AM65-52) according to an internal method on Culex pipiens.
* Field test conducted in Germany with the product VECTOBAC WG (37 % w/w Bti AM65-52) according to an internal method on four mosquitoes’s species (Aedes cantans, Aedes communis, Aedes cinerus and Aedes punctor).
* Field test conducted in France with the product VECTOBAC WG (37 % w/w Bti AM65-52) according to an internal method on Aedes caspius.
* Field test conducted in Italy with the product VECTOBAC WG (37 % w/w Bti AM65-52) according to an internal method on two mosquitoes’s species (Aedes albopictus and Culex pipiens).
* Field test conducted in Kenya with the product VECTOBAC WG (37 % w/w Bti AM65-52) according to an internal method on Anopheles gambiae.
* Field test conducted in Burkina Faso with the product VECTOBAC WG (37 % w/w Bti AM65-52) according to an internal method on Anopheles gambiae
* Field test conducted in Afghanistan with the product VECTOBAC WG (37 % w/w Bti AM65-52) according to an internal method on two mosquitoes’s species (Anopheles spp and Culex).
* Field tests conducted in Europe with other based Bti formulations according to an internal method on Anopheles spp species.

Several studies already submitted for annex I inclusion and performed with the product VECTOBAC WDG have been submitted. They were not validated as they were initially rejected by the RMS (see table of summary data in annex 9).

**Toxicology data**

No new toxicological study has been provided. VECTOBAC WG is the representative product of the CAR of the active substance.

**Residue data**

No specific residue data were submitted in the context of this dossier. The product VECTOBAC WG is intended to be applied by professional users, outdoor on landing water, including water surrounding rice and waters in irrigated crops. No data on potential exposure have been submitted however an argumentation has been proposed by the applicant:

*”Use of VECTOBAC WG is requested in water surrounding rice (into paddy water). It should be remembered the rice paddies contain stagnant water that is an important area for proliferation of mosquito larvae and contains numerous other contaminants and a large natural microbial load. However, other points of consideration is that VECTOBAC WG (as liquid application) will only be sprayed on the water when the rice plants are small, as if the vegetation is high this formulation would not be appropriate for application as the spray would partially be intercepted by the vegetation thus not reaching the targeted mosquito larvae in the water. Once the vegetation becomes more dense, applications are usually made with granular formulations that can penetrate the vegetation. Notwithstanding the information above it should also be remembered that mosquitoes proliferate in water and towards the end of the rice growing period the fields are dried approximately 4 weeks before the grain harves, thus permitting plenty of time for the Bti to degrade due to UV lightt. Rice grains are also covered by a husk that is removed prior to consumption.”*

**Ecotoxicology data**

No new studies were conducted with VECTOBAC WG. One study conducted with VECTOBAC WG on bee which was already assessed in the CAR of *Bacillus thuringiensis* subsp. *israelensis* Serotype H-14 Strain AM65-52 was re-submitted in the VECTOBAC WG dossier.

### Access to documentation

No letter of access is submitted. The applicant is the owner of all data in this dossier.

# Summary of the product assessment

## Identity related issues

The source of the *Bti* Strain AM65-52 slurry used in the biocidal product VECTOBAC WG is the same as the source used for annex I inclusion (see confidential part).

The manufacturing process is continuous. The microbial technical active substance (*Bti* Strain AM65-52 slurry) is an hypothetical stage.

The source of the *Bti* Strain AM65-52 slurry used in the biocidal product VECTOBAC WG is the same as the source used for annex I inclusion (see confidential part).

## Classification, labelling and packaging

### Classification of the active substance

No classification is need for microorganism. Therfore no classification for the active substance is needed for *Bacillus thuringiensis subsp. israelensis Serotype H-14 strain AM65-52*.

### Classification of the biocidal product VECTOBAC WG

No classification for the active substance VECTOBAC WG is needed. However, considering that all microbials should be regarded as potential sensitizers, the warning phrase “*Contains Bacillus thuringiensis israelensis, micro-organisms may have a potential to provoke sensitising reactions”* must be present in the label.

### Labelling of the biocidal product

No specific labelling language is required for the product VECTOBAC WG. However, considering that all microbials should be regarded as potential sensitizers, the warning phrase “Contains *Bacillus thuringiensis israelensis*, micro-organisms may have a potential to provoke sensitising reactions” must be present in the label.

### Packaging of the biocidal product

The packaging of the biocidal product VECTOBAC WG as deposited by the applicant is:

0.5, 5.0 kg HDPE container, or 25 kg fibre drums (no details on the composition of fibre drums were provided). No secondary packaging is claimed.

Packages used in the stability tests and commercialised: HDPE canisters (500 g).

* **Post authorisation data 2018 :**

Details regarding the composition of fibre drums were provided.

## Physico/chemical properties and analytical methods

### Active ingredient

#### Identity, origin of active ingredient

The source of the microbial active substance (*Bti* Strain AM65-52 slurry) used in the biocidal product VECTOBAC WG is the same source used for annex I inclusion (see confidential part).

Some methods were provided in the final CAR (Oct. 2010) of the microbial active substance *Bacillus thuringiensis israelensis* serotype H-14 strain AM65-52 for the identification of the microbial active substance at strain level (Benson, T. (2005) (AFLP on 300 Bt, B. Cereus, B. anthracis and *Bti* AM65-52); Lucchini et *al*., 2001).

No more data were required by the RMS in the final assessment report (May 2011).

Trade name of the active substance: Bti strain AM65-52 and ATCC-SD-1276.

#### Physico-chemical properties

The manufacturing process is continuous. Only physical and chemical properties on the formulation VECTOBAC WG were evaluated in the final CAR (Oct. 2010) of the microbial active substance *Bacillus thuringiensis israelensis* serotype H-14 strain AM65-52. See 2.3.2.

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

Analytical methods for the determination of microbial active substance *Bti* H14 AM65-52 (crystal proteins) in the VECTOBAC technical powder have already been performed and validated at EU level in the confidential part of the CAR of Sumitomo Chemical Agro Europe (Oct.2010).

**Table 2: Summary Analytical methods for the determination of microbial active substance Bti AM65-52**

|  |  |
| --- | --- |
|  | **Principle of method** |
| Technical active substance as manufactured: | Coddens, M. (1990):  Determination of crystal proteins in VECTOBAC technical powder  Protein crystals from ‘VactoBac’ Technical Powder were extracted using a method developed by Abbott Laboratories which used a mixture of salt and EDTA followed by high speed centrifugation. Analysis of the the crystal proteins in the resulting pellet were solubilised at 100°C in a mixture of Tris buffer, SDS, 2-mercaptoethanol and EDTA prior to analysis. The analysis used the PhastSystem (Pharmacia LKB Ltd.) commercial automated gel electrophoresis system, which used a 12.5 % acrylamide gel and silver staining. Optimised separation and development conditions for the commercial product (Pharmacia Technique P111 50-01-394, Pharmacia Development technique 11-B-090-02) were used without modification.  Conclusion:  Validation data were provided and were accepted by the RMS. |
| Impurities in technical active substance: | No impurity was defined in the CAR. Nevertheless, the content of beta exotoxin was determined in five batches of VECTOBAC Technical Powder in the study Isaacson, J.A. (1991) and is summarised in the CAR (Oct.2010). |
| Contaminants | Testing for microbial pathogen contaminants in VECTOBAC WDG is contained in the following Abbott Laboratories standard procedures:  STM.0309600: Standard Procedure – Coliform Enumeration & Identification From Product  The product is diluted in sterile USP phosphate buffer, plated onto violet red bile agar and incubated at 35-37°C for 20-24 hours. Coliform colonies appear dark red or purplish red in colour. If a positive response is obtained this is further investigated by Gram staining, or API 20 Enterobacteriaceae screening.  STM0323700: Standard Procedure – Salmonella Tests for Isolation & ID From Product  The product is incubated in lactose broth at 35-37°C for 24 hours then plated onto sterile brilliant green agar, xylose-lysine-desoxycholate (XLD) agar and bismuth sulphite agar and incubated for 24 hours at 35-37°C. Salmonella colonies appear as small transparent, colourless, pink or white opaque colonies surrounded by a pink to red zone in brilliant green agar, red colonies in XLD agar and black/green colonies in bismuth sulphite agar. If a positive response is obtained this is further investigated initially by Gram staining.  STM.0154800: SP - Clostridium perfringens Enumeration  The product is diluted in sterile USP phosphate buffer, plated onto oxoid perfringens agar and incubated at 35-37°C for 18-24 hours. Perfringens colonies appear black in colour. If a positive response is obtained confirmation is obtained by stab-inoculation of nitrate motility agar and lactose gelatine agar.  STM.0329100: SP – Total Aerobic Microbial Count, Pour Plate, Spread Plate, Rodac Plate  The product is diluted in sterile USP phosphate buffer, plated onto tryptic soya agar and incubated at 30-35°C for 48-72 hours. Following incubation the plates are examined for colonies.  STM.0323800: Standard Procedure – Pseudomonas aeruginosa Enumeration and ID from Product  The product is diluted in sterile USP phosphate buffer, plated onto cetrimide agar and incubated at 30-37°C for 48-72 hours. A positive response is characterised by bluish green colonies which can be confirmed by Gram staining and an oxidase test.  STM.0154600: S. Procedure – Staphylococcus aureus Tests for Enumeration and ID from Product  The product is diluted in sterile USP phosphate buffer, plated onto cetrimide agar and incubated at 30-37°C for 48-72 hours. A positive response is characterised by bluish green colonies, which can be confirmed by Gram staining and an oxidase test.  STM.0042100: Total Combined Yeast and Mould Count  The product is diluted in sterile USP phosphate buffer, plated onto Sabour and Dextrose Agar or Potato Dextrose Agar and incubated at 20-25°C for 5-7 days. Following incubation the plates are examined for yeast or mould colonies.  STM.0154700: Standard Procedure – Enterococci Screening  The method is briefly described as follows: The product is diluted in sterile USP phosphate buffer, plated onto Bacto m-enterococcus agar and incubated at 35-37°C for 1-2 days. A positive response is characterised by pink to dark maroon colonies, which can be confirmed by Gram staining and Vitek or API 20S streptococcus systems.  Conclusion:  No validation data were provided but no more data were required by the RMS. |

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

Residue analytical methods for *Bti* H14 AM65-52 are not considered necessary as no MRL and no residue definition were set for plants, food of animal origin, soil, water and air.

### Biocidal product

#### Identity, composition of the biocidal product

The biocidal product VECTOBAC WG is the representative product assessed for the inclusion of the active substance in annex 1 of directive 98/8/EC.

Code name: ABG-6511, ABG-6490

Trade name: VECTOBAC WDG

The composition of the product is confidential and is presented in a separated confidential annex. The product contains 37.4% w/w (33%-47%) of the microbial technical active substance (fermentation slurry contains 14 % (8.0-20%) of Bacillus thuringiensis subsp. Israelensis strain AM65-52 (potency between 8789 and 10623 ITU/mg).

Some co-formulates (preservatives) were removed from the formulation deposited at France level in comparison with the formulation evaluated in the CAR (Oct. 2010). These modifications (- 0.9 %) have impact on the stability of the formulation.

* Minimum biopotency: 2700 ITU/mg
* Nominal biopotency: 3000 ITU/mg
* Maximum biopotency: Post-autorisation date required.

According to the applicant, there is not a clear correlation between spore counts and biopotency, the determination of a minimum, a maximum and a nominal content in term of colony forming unit (CFU/kg) is not possible.

The RMS is agreed with the applicant that there is no correlation between spore counts and biopotency. **nevertheless, a maximum biopotency of the product should be set and is required in post registration.**

* **Post authorisation data 2018 :**

Maximum biopotency: 3800 ITU/mg

#### Physico-chemical properties

The tested product is VECTOBAC WG. *Bti* Strain AM65-52’s content in tested product is 37.4% w/w (3000 ITU/mg).

The biocidal product does not contain hydrocarbons≥ 10 %.

The biocidal product does not contain co-formulates classified H304 cat. 1 ≥10 %.

Studies have been performed on the old composition of biocidal product VECTOBAC WG. Old and current compositions are considered to be similar (0.9 % w/w difference) for physicochemical properties.

Physical and chemical properties evaluated and validated in the CAR (Oct. 2010) are summarised below:

Terrestrial application: (2.5-1000 L of water)

Minimum use concentration: 0.0125 % w/v (0.125 kg/ ha of product in 1000 L of water).

Maximum use concentration: 40% w/v (1 kg/ ha of product in 2.5 L of water).

Aerial application: (2.5- 300 L of water)

Minimum use concentration: 0.167 % w/v (0.125 kg/ha of product in 300 L of water)

Maximum use concentration: 40 % w/v (1kg/ha of product in 2.5 L of water)

**Table 3: Physical and chemical properties**

| **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 | ABG-6511, lot 60-068-BR a.i. content not specified | Fine brown granules with musty odour | Y | IIIB 2.1/01 Young, S. (2003) | 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** | The product was stored at 20°C and 25°C in the commercial packs (HDPE canister), in compliance with CropLife Monograph 17 as representative temperatures for temperate and hot climates.  Commercial packs are light resistant and air tight and a study of the effects of light and humidity is not appropriate | ABG-6511,Batches 60-068-BR 60-070-BR 60-072-BR a.i. content not specified | |  |  |  | | --- | --- | --- | |  | T0 | After 24 months at 20 °C | | Appearance | Fine brown granules with musty odour | Fine brown granules with musty odour | | Potency (ITU/mg)  (Method:  Bioassay ABT 401/024588)  60-068-BR\*:  60-070-BR\*:  60-072-BR\*: | 3973± 171  4056± 178  3465  ± 275 | 3518± 276  3506± 185  3120  ± 239 | | Wettability  (CIPAC MT 53.3) | 3 sec | 3 sec | | Dust content  (CIPAC MT 58.2)  Size range  850-250 µm  250-150µm  <150µm | 63.7 %  33.4 %  2.9 % | 63.9 %  32.8 %  3.2 % | | pH of 1 dilution  (CIPAC MT 75) | 5.85 | 5.62 | | Wet sieving on 75 µm sieve  (CIPAC MT167) | 1.6 % | 2.2 % | | Suspensibility  (CIPAC MT168)  At 0.13 g/L  At 67 g/L | 106.5 %  101.6 % | 101.4 %  101.2 % | | Dustiness (CIPAC MT171) | Nearly dust free | Nearly dust free | | Dispersibility (CIPAC MT 174)  At 10 g/L | 94.0 % | 94.4 % | | Attrition characteristic (CIPAC MT 178) | 99.6 % | 99.7 % | | Packaging (plastic screw top tubs: HDPE canisters (500 g)) | No alteration was observed | |   \*: % of change at 20 °C and after frozen is in acceptable FAO range (%).   |  |  |  | | --- | --- | --- | |  | T0 | After 24 months at 25 °C | | Appearance | Fine brown granules with musty odour | Fine brown granules with musty odour | | Potency (ITU/mg)  60-068-BR\*\*  60-070-BR\*\*  60-072-BR\*\* | T0 | After 24 months | | 3973± 171  4056± 178  3465± 275 | 3010± 295  3280± 128  2904± 313 | | Wettability  (CIPAC MT 53.3) | 3 sec | 2 sec | | Dust content  (CIPAC MT 58.2)  Size range  850-250 µm  250-150µm  <150µm | 63.7 %  33.4 %  2.9 % | 66.5 %  30.3%  3.1% | | pH of 1 dilution  (CIPAC MT 75) | 5.85 | 5.61 | | Wet sieving on 75 µm sieve  (CIPAC MT167) | 1.6 % | 2.3 % | | Suspensibility  (CIPAC MT168)  At 0.13 g/L  At 67 g/L | 106.5 %  101.6 % | 94.1 %  101.7 | | Dustiness (CIPAC MT171) | Nearly dust free | Nearly dust free | | Dispersibility at 10 g/L (CIPAC MT 174) | 94.0 % | 97.3 % | | Attrition characteristic (CIPAC MT 178) | 99.6 % | 99.7 % | | Packaging (plastic screw top tubs: HDPE canisters (500 g)) | No alteration was observed | |   \*\*: % of change at 25 °C is in acceptable FAO range (%) for 1 batch on 3.  \*\*: % after frozen is in acceptable FAO range (%).   |  |  |  | | --- | --- | --- | |  | After 2 months at -18 °C (T0) | After 24 months at -18°C | | Potency (ITU/mg)  60-068-BR\*\*  60-070-BR\*\*  60-072-BR\*\* | 3885± 563  3972± 717  3232  ± 532 | 3740± 258  3621± 220  3474  ± 306 |   \*\*: % after frozen is in acceptable FAO range (%).  Microbial contaminants:  According to the Applicant the microbial contamination within the same study should not be necessary. This is a dry product with no nutrients for microorganism to grow on. Microorganisms need water in an available form to grow in food products. The control of the moisture content even in foods is one of the oldest exploited preservation strategies. Thus product in its original packaging will not present sufficient moisture nor nutrients for microbial growth.  Effect of the light:  According to the Applicant HDPE contains light stabilizers, specifically to not react with UV light. Light stabilizers are classified as anti-aging additives and are used to protect packages and products against photo-oxidative degradation reactions caused by the UV-light to which they are exposed.  Additionally, under normal warehousing conditions, one would not expect a lot of UV to be hitting the product. Canisters are in cardboard boxes which would also protect against light. | Y | IIIB 2.2.1/01 Young, S. (2003) | Acceptable  The biopotency of the microbial active substance is near the FAO specifications before and after 24 months at 20 °C, after 24 months at 25 °C and after 24 months at -18 °C is considered acceptable.  Physical and chemical properties are considered acceptable before and after 24 months at 20 °C and after 24 months at 25 °C.  **Nevertheless, the test of the persistent foaming was not performed before and after 24 months at 20 °C and after 24 months at 25 °C and was required in post registration.**  The applicant did not determine the microbial contaminants in the formulation before and after storage, he provided an inacceptable justification.  **The determination of microbial contaminants indicated in OECD 65 “issue paper on microbial contaminant limits for microbial pest control products (Oct. 2011)”, the persistent foaming according to GIFAP n° 17 before and after optimal storage in the commercial package (HDPE) wer required in post registration.**  **As there is no accelerated storage at high temperature, it should be mentioned on the label “Do not store at temperature above 25 °C”.**  The justification of the applicant concerning the effect of light “the packaging is opaque to the light” is not acceptable.  The "opaque" term does not mean barrier to the light.  The agents added to the packaging to prevent the degradation of the packaging do not allow to make barrier from the light.  **The Applicant should indicate the following mention on the label “protect the biocidal product from the light.** |
| * **Post authorisation data – 2020 :** | The product was stored at cold temperature | VectoBac WDG  Batches 289589BJ30  And  289589BJ30 | The precision on the temperature of storage is not known.  A new test have recently started a new GLP storage stability study, which will included all the necessary phys/chem properties and bioburden according to “OECD 65 issue paper on microbial contaminant limits for microbial pest control product (Oct. 2011)”, for which we would have the two-year shelf-life results toward the end of 2022. Unfortunately, the start of the study was delayed due to the Covid-19 health crisis. | N | Tyler T., 2020 | The content of microbial contaminants before and after 2-year cold storage (4°C) is in the acceptable limits. Nevertheless, the determination of microbial contaminants before and after 2-years was required **at 25 °C** and not at 4°C . Considering the absence of the results at 25 °C the mitigation measure should be changed to: “**Do not store at temperature above 4 °C”** A study plan (storage stability study at 25 °C) was provided, on a product VBC-60782 (100 % Bti AM 65-52) which is different from  **Vectobac WG. Results, on VBC-60782 could not be used to conclude on Vectobac WG**. **Furthermore, the study plan does not include the determination of microbial contaminants at 25 °C.** |
| * **Post authorisation data – 2020 :** | Statement for the persistent foaming | / | Foam is a collection of small bubbles of air that accumulate on or near the surface of the fluid due to the properties  Thus, foam formation is an integral part of a product, that should not vary with storage time unless there is:  • Contamination: Will not occur during a storage stability study  • Depleted defoamer: While breakdown of a defoamer could occur during storage, VectoBac WG does not contain a defoamer.  Rather, VectoBac WG contains some coformulants which could cause foam. As no foaming is seen at time zero, if the formulants were to break down during storage, this would reduce the foaming potential.  In conclusion VectoBac WG made no perceivable foam at the time zero in a storage stability test. Storage at different temperature regimens will not change this property based on the formulants used in this product. | / | / | **Not acceptable**  **The amount of foam after storage cannot be predicted.**  It should be noted that a stability study plan (24 months at 25 °C) for the determination physical and chemical properties on the product VBC-60782 (100% Bti AM 65-52) was provided. **Nevertheless, as the product is different from Vectobac WG, the results of persistent foaming on VBC-60782 could not be used to conclude on Vectobac WG**. |
| **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)** | EC method A14 explosive properties Theoretical assessment | Not relevant | It can be concluded that ‘VECTOBAC’ WG is unlikely to undergo rapid decomposition with the evolution of gases or release of heat and does not therefore present a risk of explosion. | N | IIIB 2.3/01 Curl, M.G., (2005a) | Acceptable |
| EC method A17 oxidising properties Theoretical assessment | Not relevant | It can be concluded that ‘VECTOBAC’ WG will not be an oxidizer and will be capable of reacting exothermically with combustible materials. | N | IIIB 2.3/02 Curl, M.G., (2005b) | Acceptable |
| Information in these reports is confidential to Valent BioSciences and is presented in the confidential attachment under Point IIIB 2.3-01 and Point IIIB 2.3 02. | | | | |  |
| **IIIB 2.4 Flashpoint**  **and other indications of flammability(B.4.7) or spontaneous ignition (B.4.7)** | ‘VECTOBAC’ WG is not a liquid formulation and therefore flash point is not relevant.  The relative self ignition test has not been conducted on ‘VECTOBAC’ WG. This test is not relevant to WG formulations as it is not used for classification purposes.  Theoretical assessment of flammability: It can be concluded that ‘VECTOBAC’ WG will not be classified as flammable. (Curl, M.G., 2005c). | | | | | Acceptable  The formulation is not flammable and it is expected not auto-flammable at ambient temperature. |
| **IIIB 2.5 Acidity, alkalinity and pH value** | CIPAC MT75 | ABG-6511, lot 60-068-BR  a.i. content not specified | pH (1 %dilution) = 5.85 at ambient temperature  and following 24 months storage at:  20°C: pH = 5.62 (1 %dilution) 25°C: pH = 5.61 (1 %dilution) | Y | IIIB 2.5/01 Young, S. (2003) | Acceptable |
| **IIIB 2.6 Viscosity and surface tension** | ‘VECTOBAC’ WG 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** | CIPAC MT53.3 (with swirling) using standard water D | ABG-6511, lot 60-068-BR a.i. content not specified | 3 seconds. | Y | IIIB 2.7.1/01 Young, S. (2003) | Acceptable |
| **IIIB 2.7.2 Persistent foaming** | CIPAC MT47.1 at 0.1 g/L and 67 g/L using standard water D | ABG-6511, lot 60-068-BR a.i. content not specified | No foam was observed at both concentrations.  An increase of 670X the dilution rate thus gave no increase in foam what so ever. The highest dose looked at represents 17% of the minimum dilution rate. So to look at the 1kg of product/2.5L would represent an increase of 5X. While possible that some increase in foaming may occur, it is highly doubtful that this foam would exceed the 60ml that makes a study unacceptable. Additionally foam is of possible concern when a product presents a toxicological problem. This is not the case with Bti strain AM65-52. | Y | IIIB 2.7.2/01 Young, S. (2003) | Acceptable  The persistent foaming is in acceptable range at 0.01 % and at 6.7 % (w/v). As there is no effect of concentration on the persistent foaming, it should be expected an acceptable persistent foaming at the maximum use concentration 40 %w/w. |
| **IIIB 2.7.3 Suspensibility, suspension stability and dispersibility** | CIPAC MT168 Active Suspensibility  using standard water D | ABG-6511, lot 60-068-BR a.i. content not specified | 106.5% at 0.13g/L 101.6% at 67g/L  and following 24 months storage at: | Y | IIIB 2.7.3/01 Young, S. (2003) | The suspensibility is in the acceptable range at 0.013 % and at 6.7 % (w/v). Results are acceptable for the minimum use concentration 0.0125% (w/v), as the results demonstrate no effect of the concentration |
| CIPAC MT174 Dispersibility  using standard water D | ABG-6511, lot 60-068-BR a.i. content not specified | Dispersibility at 10 g/L: 94.0%  The spontaneity of dispersion is determined to show the preparation is rapidly dispersed when diluted with water. This phys-chem property, according to FAO requirements, does not require it be done at the maximum use rate | Y | IIIB 2.7.3/01 Young, S. (2003) | The dispersibility is in the acceptable limits at 1 %( w/v).  The dispersibility should be performed at the maximum use concentration (40 %w/v) and was required in post registration. See data below. |
| CIPAC MT174 Dispersibility  using standard water D | ENV-18-028 “VectoBac WDG | A sample (360 g) of test item was weighed and slowly added to the test beaker containing Standard Water D. It was immediately observed that on addition of the test item, a very thick and viscous dispersion was formed with patches of unwetted test item. Furthermore, it was evident that the test stirrer was not vigorous nor large enough to effectively mix the test item and water.  An assessment of the spontaneity of dispersion of the test item, VectoBac WDG, according to the test method CIPAC MT 174, was not feasible due to the very high application concentrations of the formulation in water.  The test item does not readily disperse using the specified test equipment and instead the product should be thoroughly mixed prior to use according to the product application labels. | Y | COMB, T., 2018 | Acceptable  The justification of the Applicant can be considered acceptable. The diluted prodcuct should be stired before and during the application. |
| **IIIB 2.7.4 Wet sieve and dry sieve test** | ‘VECTOBAC’ WG is not a dustable powder and therefore a dry sieve test to determine if the particle size is suitable is not relevant. | | | | |  |
| CIPAC MT167 Wet sieving Retention on 75 µm sieve | ABG-6511, lot 60-068-BR a.i. content not specified | 1.6% retained | Y | IIIB 2.7.4/01 Young, S. (2003) | Acceptable |
| **IIIB 2.7.5 Particle size distribution, content of dust/fines, attrition and friability** | CIPAC MT58.2 Dry sieving (equivalent to OECD 110) | ABG-6511, lot 60-068-BR a.i. content not specified | Size range: 850-250µm: 63.7% 250-150µm:33.4% <150µm: 2.9% | Y | IIIB 2.7.5/01 Young, S. (2003) | Acceptable |
| CIPAC MT171 Dustiness | ABG-6511, lot 60-068-BR a.i. content not specified | ‘Nearly dust free’ | Y | IIIB 2.7.5/01 Young, S. (2003) | Acceptable |
| CIPAC MT178 Attrition resistance | ABG-6511, lot 60-068-BR a.i. content not specified | Attrition resistance: 99.6% | Y | IIIB 2.7.5/01 Young, S. (2003) | Acceptable |
| **IIIB 2.7.6 Emulsifiability, re-emulsifiability, emulsion stability** | ‘VECTOBAC’ WG is not an emulsifiable formulation and therefore emulsion characteristics are not relevant. | | | | | Acceptable |
| **IIIB 2.7.7 Flowability, pourability and dustability** | ‘VECTOBAC’ WG is not intended for broadcasting as a dry granule using application equipment and is supplied in rigid containers. The determination of flowability following compression is not relevant for WG formulations under these circumstances. | | | | | Acceptable |
| **IIIB 2.8** | **Physical, chemical and biological compatibility with other products** | | | | |  |
| **IIIB 2.8.1 Physical compatibility** | ‘VECTOBAC’ WG 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** | ‘VECTOBAC’ WG 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** | ‘VECTOBAC’ WG 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 ‘VECTOBAC’ WG is a Wettable Granule (WG). All studies have been performed in accordance with the current requirements. The appearance of the formulation is fine brown granules with a musty 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 5.9 at ambient temperature. Stability data indicate a shelf life of at least 2 years at 20 and 25 °C (in HDPE packaging). The biocidal product is stable for 24 months at -18 °C. Its technical characteristics are acceptable for a wettable granule formulation.  The formulation is not classified for the physical-chemical part.  **The formulation must not be stored at a temperature above 25 °C during 24 months.**  **The formulation should be protecting from the light.**  **Data on the determination of microbial contaminants indicated in OECD 65 “issue paper on microbial contaminant limits for microbial pest control products (Oct. 2011)”and the persistent foaming according to GIFAP n° 17 are required before and after storage of the product at 25°C for 2 years in the commercial package (HDPE) are required in post registration.**  **The dispersibility should be performed at the maximum use concentration (40 %w/v) and is required in post registration.**  **The composition detail of other claimed packages “Fiber drums” should be provided in post registration.**   * **Post authorisation data – 16/11/2017 :**   The determination of the *Salmonella* and *Staphylococcus aureus* in five batches of the product Vectobac WG using a validated method with a limit of detection of 10 CFU/g instead of **“**absent in 25 g” and “absent in 1 g” respectively was performed and the results provided. **The confirmation of absence in 25 g for *Salmonella* and in 1 g for *Staphylococcus aureus* was required**  The composition detail of the fiber drums has been submitted and is considered acceptable.   * **Post autoriastion data – 21/07/2020; 28/10/2020; 16/12/2020 :**   A justification on the results on the dispersibility at the maximum use concentration (40 %w/v) was provided and is considered acceptable. **The product should be stired before and during application.**  The confirmation of absence of the *Salmonella* and *Staphylococcus aureus* in five batches of the product Vectobac WG using a validated method with the criteria of absence in 25 g and absence in 1 g respectively according to the document OECD 65 (oct. 2011) was not provided. Nevertheless, as the claimed uses is non-food uses and as acceptable results in 10 g of five batches of Vectobac WG are available, no more confirmatory data required.  A study for the determination of microbial contaminants according to the document OECD 65 (oct. 2011) in the same batch of the product Vectobac WG before and after 24 months at 4 °C was provided. The results are considred acceptable **but cannot be extrapolated at 25 °C. Considering the absence of the results at 25 °C the mitigation measure should be changed to: “Do not store at temperature above 4 °C” instead of “Do not store at temperature above 25°C”.**  A study of the persistent foaming before and afer 24 months at 25 °C in the same batch of the product according to FAO manual (2010) and according to the GIFAP n° 17 (2009) was not provided and remain missing.  It should be noted that a stability study plan (24 months at 25 °C) for the determination physical and chemical properties on the product VBC-60782 (100% Bti AM 65-52) was provided **Nevertheless, as the product is different from Vectobac WG, the results of persistent foaming on VBC-60782 could not be used to conclude on Vectobac WG**.  **The following data has to be submitted in the application for renewal of the product authorisation:**  A study of the persistent foaming before and after 24 months adapted to the recommended storage temperature of the product according to FAO manual (2010) and according to the GIFAP n° 17 (2009) | | | | |  |

#### Analytical method for determining the active substance and relevant component in the biocidal product

**2.3.2.3.1 Methods for microbial active substance**

The analytical method below has already been provided at EU level in the CAR (Oct. 2010) of the microbial active substance.

**Table 4: Methods for microbial active substance**

|  |  |
| --- | --- |
| **Reference** | Young, S. (2003) ‘VECTOBAC’ WG: Two year storage stability. Huntingdon Life Sciences Ltd., unpublished report no. ABT 401/024588. |
| **Data protection** | Yes |
| **Data owner** | Valent BioSciences |
| **GLP** | No |

**Principle of the method**

The assay is based on the quantal dose response of 3 days post hatch yellow fever mosquito (Aedes aegypti) to the test substance incorporated in deionised water. The percentage mortality response is obtained by weighted probit-log dose regression and is expressed as potency (ITU per mg) relative to the reference substance (containing Bacillus thuringiensis spp israelensis). 170 mg test substance was transferred to a glass bottle and deionised water added prior to shaking for 20 minutes. Dilutions were performed to give six concentrations, 2.5, 4.0, 5.5, 7.0, 8.5 and 10.0 mL active in a total volume of 10mL solution. Larvae of approximately uniform size were visually selected from the rearing pan for use in the test. Twenty larvae were added to a number of cups containing de-ionised water (90 mL) and the 10 mL of the test concentrations were added. Untreated controls consisted of deionised water and test organisms. Assays of test substance and reference substance were assayed daily. The number of live larvae was counted after 16 to 18 hours and immobile larvae were probed to check for movement.

**Results**

**Repetability:** The % RSD was calculated for each batch tested. Data are summarised in tables below.

**Table 5: Mean potency (ITU/mg) with time and temperature**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Time (months)** | **0** | **2** | **2** | **6** | **6** | **6** | **12** | **12** | **12** | **18** | **18** | **18** | **24** | **24** | **24** |
| **Temp (°C)** |  | **FR** | **25** | **FR** | **20** | **25** | **FR** | **20** | **25** | **FR** | **20** | **25** | **FR** | **20** | **25** |
| 60-068-BR | 3973 | 3885 | 3998 | 3584 | 3655 | 3434 | 3703 | 3415 | 3409 | 3575 | 3652 | 3287 | 3740 | 3518 | 3010 |
| SD | 171 | 563 | 496 | 375 | 385 | 237 | 165 | 104 | 58 | 160 | 344 | 84 | 258 | 276 | 295 |
| n | 4 | 6 | 6 | 6 | 5 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 5 | 5 | 5 |
| %RSD | 4.3 | 14.5 | 12.4 | 10.5 | 10.5 | 6.9 | 4.5 | 3.0 | 1.7 | 4.5 | 9.4 | 2.6 | 6.9 | 7.8 | 9.8 |
| m-SD | 3802.0 | 3322.0 | 3502.0 | 3209.0 | 3270.0 | 3197.0 | 3538.0 | 3311.0 | 3351.0 | 3415.0 | 3308.0 | 3203.0 | 3482.0 | 3242.0 | 2715.0 |
| m+SD | 4144.0 | 4448.0 | 4494.0 | 3959.0 | 4040.0 | 3671.0 | 3868.0 | 3519.0 | 3467.0 | 3735.0 | 3996.0 | 3371.0 | 3998.0 | 3794.0 | 3305.0 |
| % variation with T0 or T0 FR (using m-SD and m+SD) | \_ | \_ | **18.2** | **19.2** | 6.3 | -3.4 | **16.4** | -7.4 | -8.8 | **12.4** | 5.1 | **-11.3** | **20.3** | -0.2 | -13.1 |
| % variation with T0 or T0 FR (using m) | \_ | \_ | **0.6** | **-7.7** | **-8.0** | **-13.6** | **-4.7** | **-14.0** | **-14.2** | **-8.0** | **-8.1** | **-17.3** | **-3.7** | **-11.5** | **-24.2** |
| 60-070-BR | 4056 | 3972 | 4123 | 3962 | 3583 | 3800 | 3546 | 3380 | 3496 | 3522 | 3432 | 3160 | 3621 | 3506 | 3280 |
| SD | 178 | 717 | 560 | 364 | 233 | 299 | 206 | 63 | 143 | 46 | 217 | 60 | 220 | 185 | 128 |
| n | 4 | 8 | 6 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 5 | 5 | 5 |
| %RSD | 4.4 | 18.1 | 13.6 | 9.2 | 6.5 | 7.9 | 5.8 | 1.9 | 4.1 | 1.3 | 6.3 | 1.9 | 6.1 | 5.3 | 3.9 |
| m-SD | 3878.0 | 3255.0 | 3563.0 | 3598.0 | 3350.0 | 3501.0 | 3340.0 | 3317.0 | 3353.0 | 3476.0 | 3215.0 | 3100.0 | 3401.0 | 3321.0 | 3152.0 |
| m+SD | 4234.0 | 4689.0 | 4683.0 | 4326.0 | 3816.0 | 4099.0 | 3752.0 | 3443.0 | 3639.0 | 3568.0 | 3649.0 | 3220.0 | 3841.0 | 3691.0 | 3408.0 |
| % variation with T0 or T0 FR (using m-SD and m+SD) | \_ | \_ | **20.8** | **32.9** | -1.6 | 5.7 | **15.3** | **-11.2** | -6.2 | 9.6 | -5.9 | **-17.0** | **18.0** | -4.8 | **-12.1** |
| % variation with T0 or T0 FR (using m) | \_ | \_ | **1.7** | **-0.3** | **-11.7** | **-6.3** | **-10.7** | **-16.7** | **-13.8** | **-11.3** | **-15.4** | **-22.1** | **-8.8** | **-13.6** | **-19.1** |
| 60-072-BR | 3465 | 3232 | 3574 | 3528 | 3305 | 3308 | 3474 | 3227 | 3119 | 3192 | 2890 | 2849 | 3474 | 3120 | 2904 |
| SD | 275 | 532 | 308 | 242 | 387 | 338 | 51 | 123 | 113 | 117 | 248 | 58 | 306 | 239 | 313 |
| n | 4 | 8 | 4 | 4 | 6 | 5 | 4 | 4 | 4 | 4 | 4 | 4 | 5 | 5 | 5 |
| %RSD | 7.9 | 16.5 | 8.6 | 6.9 | 11.7 | 10.2 | 1.5 | 3.8 | 3.6 | 3.7 | 8.6 | 2.0 | 8.8 | 7.7 | 10.8 |
| m-SD | 3190.0 | 2700.0 | 3266.0 | 3286.0 | 2918.0 | 2970.0 | 3423.0 | 3104.0 | 3006.0 | 3075.0 | 2642.0 | 2791.0 | 3168.0 | 2881.0 | 2591.0 |
| m+SD | 3740.0 | 3764.0 | 3882.0 | 3770.0 | 3692.0 | 3646.0 | 3525.0 | 3350.0 | 3232.0 | 3309.0 | 3138.0 | 2907.0 | 3780.0 | 3359.0 | 3217.0 |
| % variation with T0 or T0 FR (using m-SD and m+SD) | \_ | \_ | **21.7** | **39.6** | **15.7** | **14.3** | **30.6** | 5.0 | 1.3 | **22.6** | -1.6 | -8.9 | **40.0** | 5.3 | 0.8 |
| % variation with T0 or T0 FR (using m) | \_ | \_ | **3.1** | **9.2** | **-4.6** | **-4.5** | **7.5** | **-6.9** | **-10.0** | **-1.2** | **-16.6** | **-17.8** | **7.5** | **-10.0** | **-16.2** |

**Table 6: Mean potency as a percentage of frozen control**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Time stored (months) temperature of storage (°C)** | | | | | | | | |
| **2/25** | **6/20** | **6/25** | **12/20** | **12/25** | **18/20** | **18/25** | **24/20** | **24/25** |
| 60-068-BR | 103 | 102 | 96 | 92 | 92 | 102 | 92 | 94 | 80 |
| 60-070-BR | 104 | 90 | 96 | 95 | 99 | 97 | 90 | 97 | 91 |
| 60-072-BR | 111 | 94 | 94 | 93 | 90 | 91 | 89 | 90 | 84 |

**Conclusion**

Validation data of the biotest provided for the determination of the biopotency of *Bti* Strain AM65-52’s in the formulation VECTOBAC WG were provided and considered as sufficient

**2.3.2.3.1 Methods for microbial contaminants**

No new method was provided. Methods for the determination of microbial contaminants in the formulation VECTOBAC WDG (ABG-6490) were provided and evaluated in the CAR (Oct. 2010). These methods follow Abbott Laboratories standard procedures and are summarized below.

STM.0309600: Standard Procedure – Coliform Enumeration & Identification From Product

The product is diluted in sterile USP phosphate buffer, plated onto violet red bile agar and incubated at 35-37°C for 20-24 hours. Coliform colonies appear dark red or purplish red in colour. If a positive response is obtained this is further investigated by Gram staining, or API 20 Enterobacteriaceae screening.

STM0323700: Standard Procedure – Salmonella Tests for Isolation & ID From Product

The product is incubated in lactose broth at 35-37°C for 24 hours then plated onto sterile brilliant green agar, xylose-lysine-desoxycholate (XLD) agar and bismuth sulphite agar and incubated for 24 hours at 35-37°C. Salmonella colonies appear as small transparent, colourless, pink or white opaque colonies surrounded by a pink to red zone in brilliant green agar, red colonies in XLD agar and black/green colonies in bismuth sulphite agar. If a positive response is obtained this is further investigated initially by Gram staining.

STM.0154800: SP - Clostridium perfringens Enumeration

The product is diluted in sterile USP phosphate buffer, plated onto oxoid perfringens agar and incubated at 35-37°C for 18-24 hours. Perfringens colonies appear black in colour. If a positive response is obtained confirmation is obtained by stab-inoculation of nitrate motility agar and lactose gelatine agar.

STM.0329100: SP – Total Aerobic Microbial Count, Pour Plate, Spread Plate, Rodac Plate

The product is diluted in sterile USP phosphate buffer, plated onto tryptic soya agar and incubated at 30-35°C for 48-72 hours. Following incubation the plates are examined for colonies.

STM.0323800: Standard Procedure – Pseudomonas aeruginosa Enumeration and ID from Product

The product is diluted in sterile USP phosphate buffer, plated onto cetrimide agar and incubated at 30-37°C for 48-72 hours. A positive response is characterised by bluish green colonies which can be confirmed by Gram staining and an oxidase test.

STM.0154600: S. Procedure – Staphylococcus aureus Tests for Enumeration and ID from Product

The product is diluted in sterile USP phosphate buffer, plated onto cetrimide agar and incubated at 30-37°C for 48-72 hours. A positive response is characterised by bluish green colonies, which can be confirmed by Gram staining and an oxidase test.

STM.0042100: Total Combined Yeast and Mould Count

The product is diluted in sterile USP phosphate buffer, plated onto Sabour and Dextrose Agar or Potato Dextrose Agar and incubated at 20-25°C for 5-7 days. Following incubation the plates are examined for yeast or mould colonies.

STM.0154700: Standard Procedure – Enterococci Screening

The method is briefly described as follows: The product is diluted in sterile USP phosphate buffer, plated onto Bacto m-enterococcus agar and incubated at 35-37°C for 1-2 days. A positive response is characterised by pink to dark maroon colonies, which can be confirmed by Gram staining and Vitek or API 20S streptococcus systems.

**Results**

Five batches analysis of VECTOBAC WDG (ABG-6490) were provided in the confidential part of the CAR (Oct. 2010), nevertheless some data are missing. Data are summarised below:

Table 7: Total Aerobic Microbial Count of five lots of VECTOBAC WDG

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Lot number** | **Dilution Factor** | **Plate count** | | **Plate count x dilution factor** | **OECD 65 limits** |
| **Col/plate** | **Average** |
| 30-060-BR | 109 | 46, 39 | 42.5 | 4.3 x 1010 | <105 CFU/g |
| 30-061-BR | 109 | 39, 46 | 42.5 | 4.3 x 1010 |
| 30-065-BR | 109 | 36, 33 | 34.5 | 3.5 x 1010 |
| 30-066-BR | 109 | 40, 51 | 45.5 | 4.6 x 1010 |
| 30-067-BR | 109 | 30, 45 | 37.5 | 3.8 x 1010 |

Table 8: Bioburden screening of four lots of ‘VECTOBAC’ WDG

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Lot number** | **Contaminant** | **Dilution Factor** | **Plate count** | | **Plate count x dilution factor** | **OECD 65 limits** |
| **Col/plate** | **Average** |
| 30-060-BR | Clostridium perfringens | 101 | 0, 0 | 0 | < 10 | - |
| Total coliform | 101 | 0, 0 | 0 | < 10 | <10CFU/g |
| Pseudomonas aeruginosa | 101 | 0, 0 | 0 | < 10 | Absent in 1 g |
| **Staphylococcus aureus** | **101** | **0, 0** | **0** | **< 10** | **Absent in 1 g** |
| Enterococci (Gp. D) | 101 | 7, 15 | 11 | 1.1 x 102 | - |
| *Vibrio* | No data provided | | | | Absent in 25 g |
| *Shigella* | No data provided | | | | Absent in 25g |
| ***Salmonella*** | **No data provided and required** | | | | **Absent in 25g** |
| **Yeast and mould** | **No data provided and required** | | | | **<1000 CFU/g** |
| **30-061-**BR | Clostridium perfringens | 101 | 0, 0 | 0 | < 10 | - |
| Total coliform | 101 | 0, 0 | 0 | < 10 | <10CFU/g |
| Pseudomonas aeruginosa | 101 | 0, 0 | 0 | < 10 | Absent in 1 g |
| **Staphylococcus aureus** | **101** | **0, 0** | **0** | **< 10** | **Absent in 1 g** |
| Enterococci (Gp. D) | 101 | 12, 19 | 15.5 | 1.6 x 102 | - |
| *Vibrio* | No data provided | | | | Absent in 25 g |
| *Shigella* | No data provided | | | | Absent in 25g |
| ***Salmonella*** | **No data provided and required** | | | | **Absent in 25g** |
| **Yeast and mould** | **No data provided and required** | | | | **<1000 CFU/g** |
| 30-065-BR | Clostridium perfringens | 101 | 0, 0 | 0 | < 10 | - |
| Total coliform | 101 | 0, 0 | 0 | < 10 | <10CFU/g |
| Pseudomonas aeruginosa | 101 | 0, 0 | 0 | < 10 | Absent in 1 g |
| **Staphylococcus aureus** | **101** | **0, 0** | **0** | **< 10** | **Absent in 1 g** |
| Enterococci (Gp. D) | 101 | 92, 78 | 85 | 8.5 x 102 | - |
| *Vibrio* | No data provided | | | | Absent in 25 g |
| *Shigella* | No data provided | | | | Absent in 25g |
| ***Salmonella*** | **No data provided and required** | | | | **Absent in 25g** |
| **Yeast and mould** | **No data provided and required** | | | | **<1000 CFU/g** |
| **30-067-BR** | Clostridium perfringens | 101 | 0, 0 | 0 | < 10 | - |
| Total coliform | 101 | 0, 0 | 0 | < 10 | <10CFU/g |
| Pseudomonas aeruginosa | 101 | 0, 0 | 0 | < 10 | Absent in 1 g |
| ***Staphylococcus aureus*** | **101** | **0, 0** | **0** | **< 10** | **Absent in 1 g** |
| Enterococci (Gp. D) | 101 | 87, 85 | 86 | 1.1 x 102 | - |
| *Vibrio* | No data provided | | | | Absent in 25 g |
| *Shigella* | No data provided | | | | Absent in 25g |
| *Salmonella* | **No data provided and required** | | | | Absent in 25g |
| Yeast and mould | **No data provided and required** | | | | <1000 CFU/g |

Table 9: Total Spore Count of five lots of VECTOBAC WDG

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Lot number** | **Dilution Factor** | **Plate count** | | **Plate count x dilution factor** |
| **Col/plate** | **Average** |
| 30-060-BR | 108 | 184, 206 | 195 | 2.0 x 1010 |
| 30-061-BR | 108 | 190, 224 | 207 | 2.1 x 1010 |
| 30-065-BR | 108 | 195, 188 | 191.5 | 1.9 x 1010 |
| 30-066-BR | 109 | 32, 37 | 34.5 | 3.5 x 1010 |
| 30-067-BR | 109 | 32, 32 | 32 | 3.2 x 1010 |

According to the Applicant, since other hygiene factors have been analysed and found at very low levels. Specifically analysing for Listeria monocytogenes was not deem required. Furthermore,

He indicates that the detremination of Vibrio and Shigella is an optional requirement and recommended ONLY if there is a high potential for contamination or if species of Vibrio and Shigella are known to naturally occur at the geographical location of the manufacturing site. The manufacturing site for Bti AM65-52 does not pose a high potential for contamination. Manufacturing, additionally, is done with treated purified drinking water.

The argumentation of the applicant can be considered acceptable for *Listeria monocytogenes*, Vibrio and Shigella.

No validation data were provided for the other microbial contaminants indicated in OCDE 65 (oct. 2011).

Nevertheless, according to the Applicant all methods used for microbial contamination and identification are USP (US Pharmacopeia).

So , validation data for aerobic microbial, coliforms*, staphylocucuss aureus* and *Pseudomonas aeruginosa* are not necessary.

**Nevertheless, the determination of *Salmonella and yeast and mould*  in five batches of the formulation VECTOBAC WG remains missing and is required according to OECD 65 is required in post registration. This determination should be performed with validated methods (at least positive controls and repeatability) or with international standard methods which are required in post registration.**

**Furthermore, *Staphylococcus aureus* should be determined in five batches with a validated method with a detection limit < 10 cfu/g according to OECD 65 (absence in 1g).**

**Conclusion**

The determination of microbial contaminants (aerobic microbial, coliforms*, staphylocucuss aureus* and *Pseudomonas aeruginosa*) was provided in an analysis of four or five batches using US Pharmacopeia methods and is considered acceptable according to OECD 65 (oct.2011).

**Nevertheless, the determination of *Salmonella and yeast and mould* in five batches of the formulation VECTOBAC WG remains missing and is required according to OECD 65 in post registration. This determination should be performed with validated methods (at least positive controls and repeatability) or with international standard methods.**

**Furthermore, *Staphylococcus aureus* should be determined in five batches with a validated method with a detection limit < 10 cfu/g according to OECD 65 (absence in 1g).**

* **Post authorisation data – 16/11/2017 :**

**Reference:** Winston Lin, Ph.D., 2015, Bioburden analysis of Vectobac WDG, Performing laboratory project: 2539 SN1, Performing laboratory: IIT Research Institute Life Sciences Group, Chicago, Illinois, Sponsor: Valent BioSciences Corporation, GLP

**Principle**

Twenty-five grams of each test substance lot was removed from its original bottle and diluted in 250mL sterile ASTM Type 1 water. To detect the bioburden level of the target microorganisms, aliquots (O.l mL) of the suspension and diluted suspension (1: 10 dilution) were plated onto selective media *(Salmonella-Shigella* Agar, modified; Mannitol Salt Agar; *Pseudomonas* Cetrimide Agar; m-Enterococcus Agar; Violet Red Bile Agar; *Listeria monocytogenes* Chromogenic Agar; and CHROMagar *Vibrio)* in duplicate. In addition, positive control microorganisms were streaked for isolation on the appropriate selective media. All plates (including the plates inoculated with the positive control target microorganisms) were examined for growth of the target microorganisms and results were recorded. To determine the total bacterial spore count of the test substance for each lot, a 2mL sample of the 1: 10 suspension was removed and placed in a heated water bath at approximately 65°C for 45 minutes. Following heat treatment, a 10-fold serial dilution was performed by diluting the test substance in ASTM Type 1 water. One tenth milliliter (0.1mL) aliquots from five select dilutions (10"7 to 10"11) were plated in duplicate on Trypticase Soy agar plates. All plates were inverted and incubated at approximately 35°C overnight (18 to 24 hours). The total spore titer of the test substance (for each lot) was determined by counting the colonies on duplicate spread plates after incubation. Titers are expressed as viable colony forming units (CFU) per gram of test substance. The qualification of the spore count methodology was performed by selecting one of the five lots, performing a 10 fold serial dilution and platting 5 dilutions in duplicate (as was done to determine the bacterial spore count) three consecutive times.

**Results**

Five batches analysis of VECTOBAC WDG were provided. Only data on salmonella, staphylococcus aureus and yeast and mould are summarised below:

Total Total bacterial spore count of five lots of VECTOBAC WDG

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Lot number** | **Dilution Factor** | **Plate count** | | **Plate count x dilution factor** |
| **Col/plate** | **Average** |
| 246209BJ30 | 108 | 55.79 | 67.0 | 6.70 x 1010 |
| 246097BJ30 | 108 | 36.44 | 40.0 | 4.00 x 1010 |
| 246096BJ30 | 108 | 42.64 | 53.0 | 5.30 x 1010 |
| 248939BJ30 | 108 | 31.21 | 26.0 | 2.60 x 1010 |
| 248940BJ30 | 108 | 32.39 | 35.5 | 3.55 x 1010 |

Bioburden screening of four lots of ‘VECTOBAC’ WDG

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Lot number** | **Contaminant** | **Dilution Factor** | **Plate count** | | **CFU/g** |
| **Col/plate** | **Average** |
| **246209BJ30** | Staphylococcus aureus | 101 | 0, 0 | 0 | **< 10** |
| *Salmonella spp./ Shigella spp.* | 101 | 0, 0 | 0 | **< 10** |
| Yeast and mould |  |  |  |  |
| **246097BJ30** | Staphylococcus aureus | 101 | 0, 0 | 0 | **< 10** |
| *Salmonella spp./ Shigella spp.* | 101 | 0, 0 | 0 | **< 10** |
| Yeast and mould |  |  |  |  |
| **246096BJ30** | Staphylococcus aureus | 101 | 0, 0 | 0 | **< 10** |
| *Salmonella spp./ Shigella spp.* | 101 | 0, 0 | 0 | **< 10** |
| **Yeast and mould** | **Not provided** | | | |
| **248939BJ30** | Staphylococcus aureus | 101 | 0, 0 | 0 | **< 10** |
| *Salmonella spp./ Shigella spp.* | 101 | 0, 0 | 0 | **< 10** |
| **Yeast and mould** | **Not provided** | | | |
| **248940BJ30** | Staphylococcus aureus | 101 | 0, 0 | 0 | **< 10** |
| *Salmonella spp./ Shigella spp.* | 101 | 0, 0 | 0 | **< 10** |
| **Yeast and mould** | **Not provided** | | | |

**Conclusion:**

The methods used for the determination of *Staphylococcus aureus* and *Salmonella* were not validated, nevertheless based on the repeatability of the results between the five batches and based on the positive controls, no more data required to validated the methods.

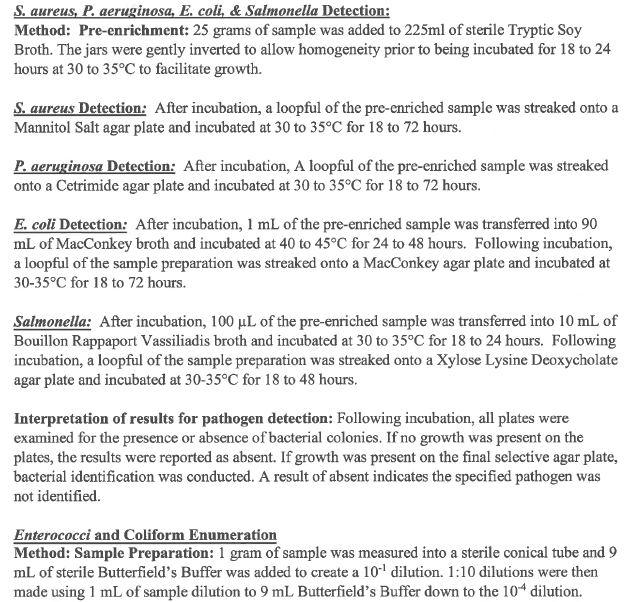
**However, the limit of quantification (<10CFU/g) of the method used for the determination of *Staphylococcus aureus* and *Salmonella* is not in accordance with the limits indicated in the documentOECD 65 (absence in 1g and absence in 25g respectively)*.* So, the determination *Staphylococcus aureus* and *Salmonella* has to be performed in five batches of Vectobac WG with a method with a detection limit in accordance with the document OECD 65 (absence in 1g for *Staphylococcus aureus and absence in 25 g for Salmonella*) . The methods used have to be validated (at least positive and negative controls and repeatability) or have to be international standard methods.**

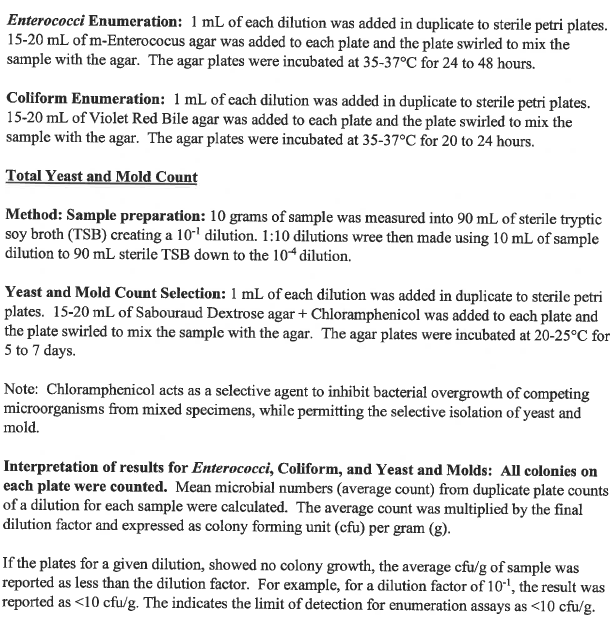
**The determination of *yeast and mould* in five batches. This determination should be performed with validated methods (at least positive controls and repeatability) or with international standard methods.**

**Post-authorisation – 2020:**

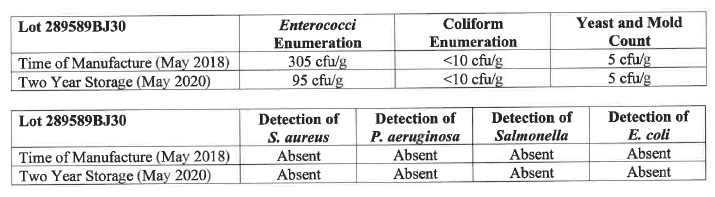
**Reference: Tyler Thome**, 2020, Microbiology Screening of Vectobac Water Dispersible Granule, Performing laboratory: Valent BioSciences Quality Control Laboratory, Osage Iowa USA, Sponsor: Valent BioSciences Corporation,

The following methods were used for the determination of microbial contaminants before and after 2-year cold storage.

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**Results**

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**Conclusion:**

Methods used for the determination of microbial contaminants in stability test can be considered similar to those used for the determination of microbial contaminants in five batches. So, they can be considered validated.

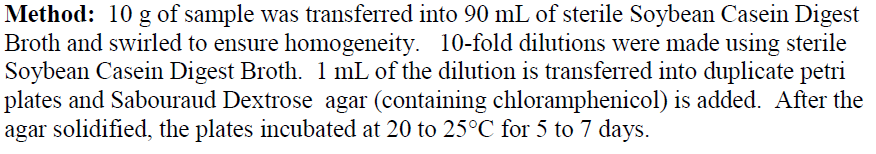
**The confirmation of the absence of *Salmonella* and *Staphylococcus aureus* in five batches of the product Vectobac WG with the criteria of absence in 25 g and absence in 1 g respectively according to the document OECD 65 (oct. 2011) was not provided. Nevertheless, as the claimed uses is non-food uses and as acceptable results in 10 g of five batches of Vectobac WG are available, no more confirmatory data were required.**

**However, data on yeast and mould were provided only on one batch. The determination of yeast and mould in five batches of the product Vectobac WG using a validated method remains missing.**

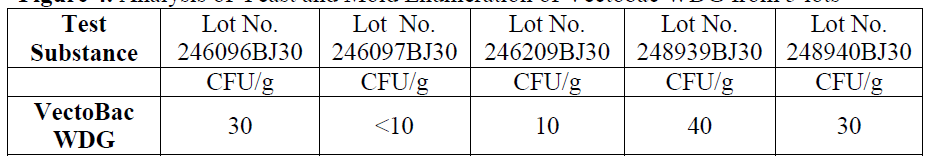
**Post authorisation March 2021:**

**Reference:** Herrero M., 2017, Yeast and Mold Screening of 5 Lots of VectoBac WDG, laboratory: Valent BioSciences Quality Control Laboratory, Osage Iowa USA, Sponsor: Valent BioSciences Corporation, not GLP.

The following method was used for the determination of yeast and mould in five batches of the product Vectobac WG:



**Results:**



**Conclusion**

Content of yeast and moulds in five batches of the product Vectobac WG was provided. Results are in accordance with the document OECD 65.

The method used for the determination of yeast and mouldwere not validated and negative control is missing. Nevertheless based on the repeatability of the results between the five batches, no more data required to validate the method.

#### 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 MRL and no residue definitions were set.

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

VECTOBAC WG is Wettable Granule (WG). It is not highly flammable, not auto-flammable at ambient temperature, not explosive and does not have oxidizing properties.

The product is stable two years at ambient temperature. Therefore, the shelf life of the product is 2 years. The product VECTOBAC WG is compatible with HDPE.

The determination of microbial contaminants according to the document OECD 65 (oct. 2011) in the same batch of the product Vectobac WG before and after 24 months at 4 °C instead of 25 °C was performed and results provided. The results are in the acceptable limits **but cannot be extrapolated at 25 °C.**

The formulation must not be stored at a temperature above ~~25~~ 4°C.

The formulation should be protected from the light.

The diluted product should be stirred before and during the application.

No data on persistent foaming were provided. The test of persistent foaing remain missing.

~~The determination of yeast and mould in five batches of the product Vectobac WG using a validated method remains missing~~.

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

* Do not store at temperatures higher than 4°C.
* Protect from the light.
* Shelf life: 2 years
* Stir the diluted product before and during the application

### Recommended methods and precautions concerning transport and fire

* Recommendation submitted by applicant and not evaluated by RMS.

***Required information linked to assessment of physico-chemical properties and analytical methods***

* A maximum biopotency of the product should be set.
* The formulation must not be stored at a temperature above 25 °C during 24 months.
* Data on the determination of microbial contaminants indicated in OECD 65 “issue paper on microbial contaminant limits for microbial pest control products (Oct. 2011)” and the persistent foaming according to GIFAP n° 17 are required before and after storage of the product at 25°C for 2 years in the commercial package (HDPE)
* The dispersibility should be performed at the maximum use concentration (40 %w/v).
* The composition detail of the fiber drums package.
* The determination of *Salmonella and yeast and mould* in five batches of the formulation VECTOBAC WG remains missing and is required according to OECD 65 . This determination should be performed with validated methods (at least positive controls and repeatability) or with international standard methods
* *Staphylococcus aureus* should be determined in post registration in five batches with a validated method with a detection limit < 10 cfu/g according to OECD 65 (absence in 1g).
* The determination of yeast and mould in five batches of the product Vectobac WG using a validated method remain missing and should be provided.
* **Post-authorisation data – 16/11/2017 :**

The maximum biopotency of the product (3800 ITU/mg) was provided and considered **acceptable**

The determination of the *Salmonella* and *Staphylococcus aureus* in five batches of the product Vectobac WG using a validated method with a limit of detection of 10 CFU/g instead of **“**absent in 25 g” and “absent in 1 g” respectively was provided and acceptable. **Nevertheless, the confirmation of absence in 25 g for *Salmonella* and in 1 g for *Staphylococcus aureus* was missing and was required**.

The detail of the specifications of packaging “Fiber drum” was provided and considered acceptable.

Every previous post-authorisation data set at the first authorisation and not provided or acceptable are still required.

* **Post-authorisation data – 2020 :**

**The following data were provided and considred acceptable:**

* an acceptable justification for the spontaneity of dispersion at the maximum use concentration (40 %(w/v)), The product should be stired before and during application.

**The following data were not provided but the data gap can be considred acceptable:**

* The confirmation of absence of the *Salmonella* and *Staphylococcus aureus* in five batches of the product Vectobac WG using a validated method with the criteria of absence in 25 g and absence in 1 g respectively according to the document OECD 65 (oct. 2011). Nevertheless, as the claimed uses is non-food uses and as acceptable results in 10 g of five batches of Vectobac WG are available, no more confirmatory data were required.
* The determination of microbial contaminants according to the document OECD 65 (oct. 2011) in the same batch of the product Vectobac WG before and after 24 months at 4 °C. The results can be considered acceptable **but cannot be extrapolated at 25 °C. Considering the absence of the results at 25 °C the mitigation measure should be changed to: “Do not store at temperature above 4 °C” instead of “Do not store at temperature above 25°C”**

**The following data were not provided and remain missing:**

* The determination of yeast and mould in five batches of the product Vectobac WG using validated methods or international standard methods according to OECD 65 (Oct. 2011).
* A study of the persistent foaming before and afer 24 months at 25 °C in the same batch of the product according to FAO manual (2010) and according to the GIFAP n° 17 (2009).

It should be noted that a stability study plan (24 months at 25 °C) for the determination physical and chemical properties on the product VBC-60782 (100% Bti AM 65-52) was provided. **Nevertheless, as the product is different from Vectobac WG, the results of persistent foaming on VBC-60782 could not be used to conclude on Vectobac WG.**

* The determination of microbial contaminants before and after 24 months at 25°C on the product Vectobac WG.
* **Post-authorisation data – March 2021 :**

**The following data were provided and considred acceptable:**

* The determination of yeast and mould in five batches of the product Vectobac WG using validated methods or international standard methods according to OECD 65 (Oct. 2011) was provided and considered acceptable.

**The following data was not provided and remain missing and should be provided at the renewal of the product:**

A study of the persistent foaming before and afer 24 months adapted to the recommended storage temperature of the product according to FAO manual (2010) and according to the GIFAP n° 17 (2009).It should be noted that a stability study plan (24 months at 25 °C) for the determination physical and chemical properties on the product VBC-60782 (100% Bti AM 65-52) was provided. **Nevertheless, as the product is different from Vectobac WG, the results of persistent foaming on VBC-60782 could not be used to conclude on Vectobac WG.**

## Effectiveness against target organisms

### Function

MG 03: Pest Control

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

VECTOBAC WG is presented as a water dispersible granule. The formulation has a potency of 3000 IUT/mg and contains 37 % w/w of the insecticidal micro-organism Bti strain AM 65-52.

The biocidal product VECTOBAC WG is a larvicide used by professional operators, for the control of mosquito larvae in water habitats (irrigation ditches, reservoirs, lakes, rivers, rice field, canals, marshland…).

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

According to the uses claimed by the applicant, the product VECTOBAC WG is intended to be used to control mosquito larvae (L1 to L4 stage). The target organisms to be controlled are *Culex*, *Culiseta*, *Anopheles* and *Aedes* (*Ochelerotatus*) genus.The specific use is the control of mosquitoes in water such as: irrigation ditches, reservoirs, lakes, rivers, canals, marshland, ponds, catch basins, drainage and roadside ditches, water in irrigated crops, waste water, sewage effluent/lagoons, septic ditches, animal waste lagoons, natural/manmade containers.

The application rates recommended by the applicant are the following:

The product VECTOBAC WG may be applied to any water where mosquitoes breed, at application rates ranging from 0.125 – 1 kg/ha (expressed also as 2.7 1012 – 1.8 1013 CFU/ha, 4.5 108 – 3 109 ITU/ha), depending on the population density and water quality. Under optimal conditions with low population densities, the lowest dose rates provide adequate control of 1st through early 4th instar larvae under most condition. In case of predominance of 4th instar larvae, high population densities, water containing high levels of organic matter, colder temperatures, higher rates should be used to provide a good control of mosquitoes.

VECTOBAC WG may be applied using ground or aerial application equipment. VECTOBAC WG is first added to water to prepare a final spray mixture. The volume of water used is dependent of the type of application equipment. Backpack and compressed air sprayers may be agitated by shaking after adding the product VECTOBAC WG to the water in the sprayer. VECTOBAC WG suspends readily in water and will say suspended over normal application periods. Brief recirculation may be necessary if the spray mixture has sat for several hours of longer.

### Effects on target organisms and efficacy

The submitted studies to demonstrate efficacy of the product VECTOBAC WG according to the uses and doses claimed, are described below. These studies were carried out with the product VECTOBAC WG (37% w/w *Bti* AM65-52) or the product VECTOBAC WDG (37% w/w *Bti* AM65-52). The latter was considered as similar to the product VECTOBAC WG, as only a preservative component was added and cross-reading is acceptable.

* Laboratory tests

## Laboratory test according to WHO 2005 method with the product VECTOBAC WG (37 % w/w *Bti* AM65-52) on *Anopheles sp*.

The second and third stage larvae of *Anopheles spp* species were in contact with the product VECTOBAC WG at rates of 0.125, 0.25, 0.5 and 1 ppm. The number of dead and live larvae was assessed in each container 24 and 48 hours after application.

🡪 The VECTOBAC WDG showed mortality greater than 90 % at the rates of 0.125, 0.25, 0.5 and 1 ppm after 48 hours of application.

## Laboratory test with the product VECTOBAC WG (37 % w/w *Bti* AM65-52) according to an internal method on *Anopheles gambiae*.

The *Anopheles gambiae* larvae in third stage were in contact with the product VECTOBAC WG. Larvae were not fed during the experiments and the mortality was scored after 24h.

The product VECTOBAC WDG showed a mortality greater than 90 % at the rate of 0.2 ppm after 24 hours of application.

* Semi-field tests

## Semi-field test conducted in Greece with the product VECTOBAC WG (37 % w/w *Bti* AM65-52) according to an internal method on *Culex pipiens*.

A simulated field trial was conducted using four rates of VECTOBAC WDG applied by spray on a mixed age population of *Culex pipiens* (stages L2/L3) larvae in a simulated rice field at rates of 125, 250, 500 and 1000 g/ha. The application was performed with a boom sprayer atomiser, with a water volume of 400 l/ha.

🡪 The VECTOBAC WDG showed a mortality greater than 90 % at the rate of 125 g/ha, 250 g/ha, 500 and 1000 g/ha after 24 hours of application.

Field tests

## Field test conducted in USA with the product VECTOBAC WDG (37 % w/w *Bti* AM65-52) according to an internal method on four mosquitoes’s species (*Aedes vexans*, *Culiseta annulata*, *Ochlerotatus sticticus* and *Aedes rossicus*).

Three mixtures were prepared using 10 litres of tap water mixed with 100, 200 and 400 g of VECTOBAC WDG, respectively, each for 1 ha. A fourth mixture was prepared using 5 litres of tap water mixed with 400 g of VECTOBAC WDG to treat 1 ha. These mixtures were then used to produce granules ice. The granules were applied via an insulated bucket equipped with a rotating device (seeder) and operated by the pilot in a Bell 47 helicopter.

First treatment site: 10 kg granules ice /ha containing 100 g VECTOBAC WDG

Second treatment site: 10 kg granules ice /ha containing 200 g VECTOBAC WDG

Third treatment site: 10 kg granules ice /ha containing 400 g VECTOBAC WDG

Fourth treatment site: 5 kg granules ice /ha containing 400 g VECTOBAC WDG

🡪 The VECTOBAC WDG showed a mortality greater than 90 % at the rates of 200 g/ha and 400 g/ha of product in 10 kg, and in 5 kg of granules ice /ha after 48 hours of application.

## Field test conducted in Spain with the product VECTOBAC WG (37 % w/w *Bti* AM65-52) according to an internal method on *Aedes* (*Ochlerotatus*) *caspius*.

A field trial was conducted using two rates of the product VECTOBAC WDG. The test materials were applied to a tidal swamp area at rates of 500 and 1000 g/ha. The application was made with a manual backpack sprayer, using a volume of 100 L/ha. Effects on a natural population of *A. caspius* larvae were observed 24 and 48 hours after application.

🡪 The VECTOBAC WDG showed a mortality greater than 90 % at the rates of 500 g/ha and 1000 g/ha after 24 hours of application.

## Field test conducted in Poland with the product VECTOBAC WDG (37 % w/w *Bti* AM65-52) according to an internal method on *Culex pipiens*.

A field trial was conducted using 3 rates of VECTOBAC WDG. The test material was applied to an irrigation ditch area in Poland using a hand operated compression sprayer at rates of 200, 400 and 800 g/ha. Effects on the natural population of *Culex pipiens* larvae were observed for 14 days after application.

🡪 The VECTOBAC WDG showed a mortality greater than 90 % at the rates of 200 g/ha after 7 days, 400 g/ha after 4 days and 800 g/ha after 2 days of treatment.

## Field test conducted in Germany with the product VECTOBAC WG (37 % w/w *Bti* AM65-52) according to an internal method on four mosquitoes’s species (Aedes cantans, Aedes communis, Aedes cinerus and Aedes punctor).

Woodland pools infested with naturally occurring mosquito larvae (*Aedes* genus) were treated with the product VECTOBAC WDG at the rates of 125, 250 and 500 g/ha.Treatments were made to which the appropriate pre-determined amount of VECTOBAC® WG had been added. This was distributed on the surface of the pool by a 1 litre hand pump. At each sampling site the larvae was counted by stage (L1/2, L3 and L4) at pre-treatment and at 24, 48, 72, 96, 120, 144 and 168 hours after treatment.

🡪 The VECTOBAC WDG showed a mortality greater than 90 % after 144 hours at 125 g/ha, after 96 hours at 250 g/ha and after 24 hours of treatment at 500 g/ha.

## Field test conducted in France with the product VECTOBAC WG (37 % w/w *Bti* AM65-52) according to an internal method on Aedes caspius.

A field trial was conducted using two rates of VECTOBAC WDG. The test material was aerially applied using an airplane equipped with boom and nozzle system at rates of 750 and 1.0 kg/ha for VECTOBAC WG. Effects on a mixed age population of *A. caspius* larvae were observed 24 and 48 hours after application.

🡪 The VECTOBAC WDG showed a mortality greater than 90 % at the rate of 1000 g/ha after 24 hours of treatment.

## Field test conducted in Kenya with the product VECTOBAC WG (37 % w/w *Bti* AM65-52) according to an internal method on *Anopheles gambiae*.

A field trial was conducted on *Anopheles gambiae* larvae (L3). The product was applied using hand held sprayer at rates of 200, 400, 600, 800 and 1600 g/ha of VECTOBAC WDG.

🡪 The product VECTOBAC WDG showed a mortality greater than 90 % at the rates of 800 and 1000 g/ha after 24 hours of treatment.

## Field test conducted in Burkina Faso with the product VECTOBAC WG (37 % w/w *Bti* AM65-52) according to an internal method on *Anopheles gambiae*

A field trial was conducted on *Anopheles gambiae* larvae (L1 to L4). The product was applied using hand held sprayer at rates of 200, 400, 800 and 1000 g/ha of VECTOBAC WDG.

🡪 The product VECTOBAC WDG showed a mortality greater than 90 % at the rates of 400, 800 and 1000 g/ha after 24 hours of treatment.

## Field test conducted in Afghanistan with the product VECTOBAC WG (37 % w/w *Bti* AM65-52) according to an internal method on two mosquitoes’s species (*Anopheles spp* and *Culex*).

A field trial was conducted on *Anopheles spp* and Culex spp larvae (L1 to L4). The product was releaseed in fine-spray modus (fire trunk) in order to allow generation of fine droplets for maximum distribution of the product on the water surface.

🡪 The VECTOBAC WDG showed a mortality greater than 90 % at the rate of 1900 g/ha after 5 days of treatment.

Three other field studies were conducted in Europe on the genus *Anopheles but* carried out with other formulations based on *Bti* AM65-52, were considered as additional data, supporting the efficacy of the active susbtance Bti on *Anopheles spp*.

In some other semi-field or field trials, performed either for 4 of them with a sprayer (Martignani B-748) mounted on a 4x4 pickup vehicle, either for 2 others with a boom sprayer atomiser, efficacy of the product VECTOBAC WG has been assessed according to the application distance and and the flow rates of the equipement , in order to evaluate the impact of these parameters on the efficacy of the treatment. Results show that both the application distance and the volume of the treatment depend a lot from the equipment used and from the area of application, and that it is necessary to ensure the calibration of the equipement.

Based on these efficacy data, the product VECTOBAC WG (37 % w/w Bti AM65-52), formulated as a water dispersible granule, at application rates ranging from 0.125 – 1 kg/ha for volumes of water/ha between 2.5 and 1000 L by terrestrial or aerial spraying, has showed an efficacy against larvae of mosquitoes (*Culex,* *Culiseta*, *Aedes* and *Anopheles genus*).

All efficacy studies are presented in annex 9.

Table 10: Summary of validated efficacy data

|  |  |  |  |
| --- | --- | --- | --- |
| **Target Organisms** | **Rates and uses acceptable** | **Method of application** | **Time delay for the biocidal action** |
| Mosquito  *genus Culex, Culiseta*  *genus Aedes* (*Ochlerotatus*)  *genus Anopheles*  from 1st larval stage to earlier 4th stage | 0.125 – 1 kg/ha  The dose rates may be depend on the population density and water quality. The lowest dose rates provide adequate control of 1st through early of 4th instar larvae. in cases of predominance of 4th instar larvae, high population densities, water containing high levels of organic matter, colder temperatrure, and / or significant water exchange, higher rates should be used to provide good control of mosquitoes. | VECTOBAC WG may be applied using ground (portable pump pressure sprayer, motorized portable blower, vehicle mounted motorized sprayer equipment) or aerial application equipment (airplane or for ice granules using a granule spreader in helicopter).  The product must be dispersed in water prior to application. The volume of water varies between 2.5 and 1000 L | from 24 hours |

### Mode of action including time delay

*Bti* Strain AM65-52 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 AM65-52 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*[[4]](#footnote-4)*

Concerning *Bacillus thuringiensis var. israelensis*, it was stated a resistance detected in a natural population of *Culex pipiens* of New York. (Paul, Harrington, Zhang, & Scott, 2005). 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) (Goldman and al., 1986, Georghiou and Wirth, 1997, Saleh and al., 2003, Mittal and al., 2005). 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 and simulies 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 (Shod and al., 1995; Abdullah and al ., 2006). Furthermore, the toxin Cyt is a key element of Bti, known to slow down the appearance of resistance to toxins Cry (Wirth and al ., 2004; Wirth and al ., 2005).

Finally, *Bti* presents a low residual activity (few days), thus can be qualified as having low environmental persistence (Lacey, 2007). However, spores of Bti are found during several weeks to several months after a treatment. The bacteria is considered as few competitive compared with the other soil bacteria, the recycling of Bti is considered as unlikely (Sheeran and Fisher, 1992; Lacey, 2007).

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 (Dupont and Boisvert, 1986; Boisvert and Boisvert, 1999; of Melo-Santos and al ., 2009; Shaheen and al ., 2010). 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 (David and al ., 2000; David and al ., 2002). This toxicity, is mainly due in fact to the presence of Bti, displayed by bacterial spreadings and by the presence of coding genes for toxin of Bti (Tilquin and al ., 2008). 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 (Paris and al ., 2011b). 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 persistance / 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 CA) validated that :

The product VECTOBAC WG (37 % w/w *Bti* AM 65-52), at application rates ranging from 0.125 – 1 kg/ha has shown a sufficient efficacy for the control of mosquitoes larvae of *Culex spp*, *Culiseta spp*, *Anopheles spp* and *Aedes spp* (*Ochlerotatus spp*). The product may be applied using ground or aerial application equipment. The lowest dose rates provide adequate control during the 1st to the early 4th larval instar. In case of predominance of 4th instar larvae, high population densities, water containing high levels of organic matter, colder temperatures, higher rates should be used to provide a good control of mosquitoes.

### Summary of efficacy assessment

The efficacy level of the product VECTOBAC WG (37% w/w *Bti* AM 65-52) is satisfactory for the uses proposed in the table 3.1.1 below.

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

To ensure a satisfactory level of efficacy, 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.

***Required information linked to efficacy assessment***

* The authorization holder has to report any observed resistance incidents to the Competent Authorities (CA) or other appointed bodies involved in resistance management.

## Description of the intended use(s)

The biocidal product VECTOBAC WG 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…). The product is efficient against larvae of mosquitoes *Culex*, *Culiseta*, *Aedes* and *Anopheles* genus. The product VECTOBAC WG may be applied using ground or aerial application equipment, with quantities of water (2.5 – 500 L / ha) sufficient to provide uniform coverage of the target area.

VECTOBAC WG is first added to water to prepare a final spray mixture. The volume of water used is dependent of the type of application equipment. Backpack and compressed air sprayers may be agitated by shaking after adding the product VECTOBAC WG to the water in the sprayer. VECTOBAC WG suspends readily in water and will say suspended over normal application periods. Brief recirculation may be necessary if the spray mixture has sat for several hours of longer.

The product should be applied during the 1st to the early 4th larval instar. The maximum number of application is 8 of 7 days interval between applications.

Table 11: 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 by professionals against larvae of mosquitoes of 1st through early 4th instar larvae of mosquitoes (*Culex*, *Culiseta*, *Aedes* and *Anopheles genus*) | VECTOBAC WG (37 % w/w *Bti* AM65-52)  application rates ranging from 0.125 – 1 kg/ha |

## Risk assessment for human health

### Hazard potential

#### Toxicology of the active substance

The toxicology of the active substance was examined extensively according to standard requirements. The results of this toxicological assessment can be found in the CAR. The following corresponds to the summary of mammalian toxicity in the assessment report of Bti.

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 following definition of a substance of concern set in the TNsG on data requirement chapter 4 (2000), “the substance is regarded as a substance of concern if [...] it is classified as dangerous **and** its concentration in the product exceeds the classification limit set in the Council Directive 88/379/EEC, as amended by Directive 1999/45/EC, for a particular dangerous property **or** the other classification limit indicated for the substance in a preparation set in Annex I of Council Directive 67/548/EEC **or** causes that the overall sum of the concentrations of dangerous substances in the product exceeds the limit for classification of the preparation set in Council Directive 88/379/EEC, as amended by Directive 1999/45/EC, for a particular dangerous property”, VECTOBAC WG does not contain any substance of concern.

#### Toxicology of the biocidal product

The toxicology of the biocidal product was examined appropriately according to standard requirements. The product was 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”*

##### Percutaneous absorption

Not relevant for micro-organisms.

##### Acute toxicity

The following information is issued from the revised draft final CAR (February 2011).

The oral LD50 of VECTOBAC WG, was determined to be greater than 5000 mg/kg bw in rats.

The dermal LD50 of VECTOBAC WG, was determined to be greater than 5000 mg/kg bw in rats.

Rats exposed to a respirable atmosphere containing the maximum achievable concentration of 0.014 mg VECTOBAC WG/L (4h) tolerated the exposure without adverse effect. The acute inhalation LC50 of the test material is greater than the maximum achievable dose level of 0.014 mg/L (4h) when administered undiluted as an aerosol to albino rats.

##### Irritation and corrosivity

*The following information is issued from the revised draft final CAR (February 2011).*

*The test material produced transient slight or well defined erythema in rabbits which persisted for up to 72 hours after dosing. Very slight oedema was also briefly observed in one animal. The primary irritation index was 0.63. VECTOBAC WG was not considered to be irritant according to EU classification guidance.*

*Transient slight conjunctival irritation was apparent in all rabbits within an hour of dosing. The severity of the response was unaffected by rinsing the eyes immediately after instillation. All reactions had resolved within 24 to 48 hours of dosing. VECTOBAC WG is not considered an ocular irritant. None of the mean scores exceeded EU classification requirements.*

##### Sensitisation

The following information is issued from the revised draft final CAR (February 2011).

The formulated product VECTOBAC WG, conducted according to Maximization test guidelines, was not considered a sensitizer. Following primary challenge using VECTOBAC Technical Powder, (Code 43494), as a 5% w/v formulation in distilled water, the incidence of grade 1 responses or greater produced in the test group (10 of 20) was compared to that of the naive control group (0 of 10). The incidence and severity of the responses in the test group were substantially greater than those produced by the naive control group indicating that sensitization had been induced. A rechallenge was conducted to investigate the elicitation of responses at a lower level. Following rechallenge using VECTOBAC Technical Powder, (Code 43494), as a 0.5% w/v formulation in distilled water, there were no grades of 1 produced. The incidence of grade ± responses produced in the test group (17 of 20) was compared to that of the naive control group (5 of1 0). The incidence and severity of the responses in the test group were slightly, but not significantly, greater than those produced by the naive control group. Although the test animals exhibited this increased incidence of ± reactions over that of their respective control group, the failure of anyone test animal to exhibit a reaction which exceeded the most severe control reaction indicates that sensitization responses had not been elicited at this level.

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 Xi, R43 unless the sensitisation response can unequivocally be attributed to a specific chemical co-formulant. Based on the negative Maximisation assay performed with VECTOBAC WG, the product is not classified as Xi, R43. However, since the product contains a micro-organism, VECTOBAC WG should carry the default precautionary statement: “*Contains Bacillus thuringiensis israelensis strain AM65-52, micro-organisms may have a potential to provoke sensitising reactions*”.

##### Other studies

No other study has been provided.

### Human exposure assessment

VECTOBAC WG contains 37.4 % w/w of the fermentation slurry containing 14 % of *Bti* strain AM65-52.

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

Table 12: Identification of main paths of human exposure towards active substance from its use in biocidal product

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **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 (portable pump pressure sprayer, motorized portable blower, vehicle mounted motorized spray 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, spraying and cleaning of spraying equipment. 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, VECTOBAC WG 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 VECTOBAC WG. 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 VECTOBAC WG as biocidal product applied with an aerial equipment. This is also considered applicable when VECTOBAC WG is applied with a vehicle mounted motorized spray equipment taking into account the expected high spray drift. Therefore, application with a portable sprayer should be envisaged for the treatment of areas closed to habitations.

Finally, in case of re-entry after treatment on rice, it is recommended to workers to wear a working 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.

Considering the intended uses and the recommended PPE, the risk for professionals is considered acceptable. However, VECTOBAC WG 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

Since bystanders are excluded from treated areas, the risk is thus considered minimal.

After aerial application, the risk for residents is considered low if drift buffer zone of 50 m is respected.

Finally, in case of re-entry after treatment on rice, it is recommended to workers to wear a working coverall and gloves. In this context, the risk is considered low.

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

No specific residue data were submitted in the context of this dossier. The product VECTOBAC WG is intended to be applied by professional users, outdoor on standding water, including water surrounding rice and waters in irrigated crops. No data on potential exposure have been submitted however an argumentation has been proposed by the applicant:

*”Use of VECTOBAC WG is requested in water surrounding rice (into paddy water). It should be remembered the rice paddies contain stagnant water that is an important area for proliferation of mosquito larvae and contains numerous other contaminants and a large natural microbial load. However, other points of consideration is that VECTOBAC WG (as liquid application) will only be sprayed on the water when the rice plants are small, as if the vegetation is high this formulation would not be appropriate for application as the spray would partially be intercepted by the vegetation thus not reaching the targeted mosquito larvae in the water. Once the vegetation becomes more dense, applications are usually made with granular formulations that can penetrate the vegetation. Notwithstanding the information above it should also be remembered that mosquitoes proliferate in water and towards the end of the rice growing period the fields are dried approximately 4 weeks before the grain harves, thus permitting plenty of time for the Bti to degrade due to UV lightt. Rice grains are also covered by a husk that is removed prior to consumption.”*

The product VECTOBAC WG is intended to be applied by spray and aerial applications.

FR is of the opinion that the argumentation of the applicant is acceptable and can be considered to support the use of VECTOBAC WG in water surrounding rice, with a pre harvest interval of 1 month. However, no data nor justification have been given for waters in irrigated crops. As indirect exposure via food cannot be excluded in those cases and without further information, application of VECTOBAC WG in waters in irrigated crops is not supported.

In Annex 8 “Residue behaviour”, the results of the residue assessment are laid out. Open literature data have been 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. VECTOBAC WG should not be applied in waters in irrigated crops, except in water surrounding rice for which a pre harvest interval of 1 month is required. Regarding consumer health protection, there are no objections against the intended uses.

#### 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 VECTOBAC WG with ground or aerial equipment when appropriate PPE were worn.

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 working coverall and gloves are worn in case of re-entry after treatment on rice.

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. VECTOBAC WG should not be applied in waters in irrigated crops, except in water surrounding rice for which a pre harvest interval of 1 month is required.

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

* Professionals must wear gloves, working coverall , goggles and respiratory mask (with P3 filter)
* VECTOBAC WG 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 users are not permitted in area being treated.
* A drift buffer zone of 50 m should be respected after aerial application and when the product is applied with a vehicle mounted motorized spray equipment. Another mode of application (such as with a portable sprayer) should be used in areas closed to habitations.
* In case of re-entry after treatment on rice, it is recommended to workers to wear a working coverall and gloves.
* VECTOBAC WG should not be applied in water surrounding crops, except in water surrounding rice for which a pre harvest interval of 1 month is required.

***Emergency***

* Not evaluated by Anses.
* Wear suitable protective clothing during handling the product. Avoid contact with skin, eyes and respiratory tract. During treatment, wear suitable protective clothing. Do not eat, drink or smoke during application and until your hands have been washed.
* Advice to doctor: symptomatic treatment is advised.

***Disposal considerations***

* None

## Risk assessment for the environment

The summary of information about the active substance *Bacillus thuringiensis* subsp. *israelensis* Serotype H-14 Strain AM65-52 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.

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

(*Summary of the information of each active substance*)

#### Degradation

##### Abiotic degradation

###### Hydrolysis in function of pH

Not applicable.

###### Photolysis in water

Not applicable

###### Photolysis in soil

Not applicable

###### Photodegradation in air

Not appicable

##### 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 for *Bacillus thuringiensis* subsp. *israelensis* Serotype H-14 Strain AM65-52 in aquatic compartment.

Table 13: 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 15. 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. This bibliographical analysis includes references proposed by the applicant. 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 highest intended doses for the product used in the studies are reminded in the table 12. For each of them, a maximum of 8 applications are intended in the product authorization dossier. 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 13, 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 14: Highest doses intended in the authorization product dossiers for the products use in the bibliographical studies

|  |  |  |
| --- | --- | --- |
| Product name | Highest intended doses | |
| VectoBac G | 15 kg/ha | 3.0E+09 ITU/ha |
| VectoBac WG | 1 kg/ha | 3.0E+09 ITU/ha |
| VectoBac 12AS | 2.5 L/ha | 3.21E+09 ITU/ha |

Table 15: 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 | VectoBac G, 12 kg/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 | Vectobac probably 12AS (1200 ITU/mg) 2-8L/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 | Vectobac 12AS, 1.5 3 and 8 L/ha, one application | Modification of population dynamic for one year for the highest dose |
| Le Goff et al., 2009 | West of France | 2 years | Vectobac 12AS (0.5L/10L 6.6 kg/ha considering depth of 20 cm, e.g. approximatively the highest depth recorded)  Vectobac WG (0.4 kg/12L, i.e. 6.6 kg/ha considering depth of 20cm)  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 | VectoBac WG, 0.2-0.3 kg/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 | VectoBac WG, 0.8-1 kg/ha, 1-2/ year | No observed effect, high impact of climatic conditions |
| Caquet et al. 2011 | West of France | 2 years | VectoBac 12AS, 0.5L/ha, or Vectobac WG 0.4 kg/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 Vectobac 12AS 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 | Vectobac 12AS, 0.8 and 2.5L/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 | Vectobac 12AS, 0.8 and 2.5L/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 | Vectobac 12AS, 2.5L/ha, 1 application | No effect on density of *Daphnia magna* and *Daphnia pulex* |
| Persson Vinnersten et al., 2010 | Sweden | 6 years | Vectobac G, 13-15 kg/ha, no information dealing with the number of applications | No effect on invertebrates |
| Lundström et al., 2010 a and b | Sweden | 6 years | Vectobac G, 15 kg/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? | Bactimos PP, Vectobac TP, Vectobac 12AS, Vactobac G, *Bti*-sand granules  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 | Vectobac 12AS, 2.5L/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). |

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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 VECTOBAC WG 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 VECTOBAC WG 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 VECTOBAC WG 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.

#### 

Table 16: 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 |

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:

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 used for risk assessment

Table 17 : Summary of PNECs of the active substance Bacillus thuringiensis subsp. israelensis Serotype H-14 Strain AM65-52 used for risk assessment

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| 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

The summary of information about the active substance *Bacillus thuringiensis* subsp. *israelensis* Serotype H-14 Strain AM65-52 is carried out with the data from the CAR of *Bacillus thuringiensis* subsp. *israelensis* Serotype H-14 Strain AM65-52 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.

No new studies were conducted with VECTOBAC WG. One study conducted with VECTOBAC WG on bee which already was assessed in the CAR of *Bacillus thuringiensis* subsp. *israelensis* Serotype H-14 Strain AM65-52 was re-submitted in the VECTOBAC WG dossier.

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

##### Aquatic organisms

Refer to section 28.2.1.1

##### Sediment dwelling organisms

Refer to section 2.8.2.1.2

##### STP micro-organisms

Refer to section 2.8.2.1.1

#### Atmosphere

Refer to section 2.8.2.2

#### Terrestrial compartment

Refer to section 2.8.2.3

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

Refer to section **2.8.2.5**

#### Summary of PNECs

Refer to section 2.8.2.4

### Environmental exposure assessment

#### Assessment of exposure to the environment

In the Annex 1 CAR, the risk assessment for the environment of Bacillus thuringiensis subsp. israelensis Serotype H-14 Strain AM65-52 has been carried out considering VECTOBAC WG as representative product. Same highest application rates are intended than in the Annex 1 CAR (0.25 - 1 kg/ha) however lower rate are also proposed for the authorization of VECTOBAC WG (0.125 kg/ha). Same active substance concentrations (374 g/kg) are reported in the Annex 1 CAR and in the product dossier. The nominal potency (3000 ITU/mg) has been confirmed by the applicant. However it should be noted that, despite a 5 batch tests indicating variation up to 4000 ITU/mg, no maximum potency has been provided. Additionally, the CFU content in VECTOBAC WG is not provided neither in the product dossier nor in the CAR but can be derived from the intended doses (1.8 E+13 CFU/ha corresponding to 1.8 E+13 CFU/kg VECTOBAC WG).

In the Annex 1 CAR, environmental exposure resulting from a ground application in standing water (ditches, pools, pastures, water retention areas, salt marshes and standing water in crops fields) were determined considering an application on a water body of 30 cm of depth. Ground applications in higher volume of water (lake) or in flowing water (rivers, canals...) are intended for the product authorization of VECTOBAC WG. It is nevertheless assumed that these additional intended uses are covered by those assessed in the Annex 1 CAR. Indeed, with a considered water depth of 30 cm, assessment of exposure of surface water for small standing water appears as a worst case compared to dilution that occur in lake and river. Acceptable risks were determined in the Annex 1 CAR, however, mistakes occurred in the PNEC and PEC derivation for soil compartment and a refinement of the calculated exposure was necessary to lead to acceptable risk for this compartment. Same calculations, than in the Annex 1 CAR, have been reported for the aquatic compartment.

Aerial applications, which are not been assessed in the Annex 1 CAR, are intended for VECTOBAC WG and have been therefore assessed.

#### Intended use 1: ground application

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

**Surface water and sediment**

VECTOBAC WG is directly applied to surface water with a maximum of 8 applications, with at least 7 days between each application. Calculations have been performed for the highest intended dose (1kg/ha; 1.8E+13 CFU/ha, 3E+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 depthand dissipation between two applications (DT50 = 50 days for spores and DT50 = 14 days for toxin, degradation constant = e(-ln(2)/DT50\*7).

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\*7)

and

PECSW, final, n = PECSW, init, n xe(-ln(2)/DT50\*7)

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* AM65-52 initial population density in water considering dilution and adsorption before any dissipation (CFU/L)

EEDSW, init, n: *Bti* AM65-52 population density in water following n applications (CFU/L)

EEDSW, final, n: *Bti* AM65-52 population density in water after 7 days following n applications (CFU/L)

PECSW, t=0: *Bti* AM65-52 initial biopotency in water considering dilution and adsorption before any dissipation (ITU/L)

PECSW, init, n: *Bti* AM65-52 biopotency in water following n applications (ITU/L)

PECSW, final, n: *Bti* AM65-52 biopotency in water after 7 days following n applications (ITU/L)

EEDSed, t=0: *Bti* AM65-52 initial population density in sediments (CFU/g)

EEDSed, final, n: *Bti* AM65-52 population density in sediments after 7 days following n applications (CFU/g)

EEDSed, init, n+1: *Bti* AM65-52 population density in sediments following n+1 applications (CFU/g)

PECSed, t=0: *Bti* AM65-52 initial biopotency in sediments (ITU/g)

PECSed, final, n: *Bti* AM65-52 biopotency in sediments after 7 days following n applications (ITU/g)

PECSed, init, n+1: *Bti* AM65-52 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 18: Inputs and defaults for step 2 water and sediment calculations

|  |  |  |  |
| --- | --- | --- | --- |
| **Constant** | **symbol (unit)** | **Value** |  |
| Application number | - | 8 | S |
| Interval between applications | (days) | 7 | S |
| Application rate | rate (CFU/ha) | 1.8E+13 | S |
| Application rate | Rate (ITU/ha) | 3E+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* AM65-52 density and biopotency in water are reported in the table below

Table 19: predicted *Bti* AM65-52 density and biopotency in water after ground application of 1kg/ha of VECTOBAC WG at surface water

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **1 application** | | **8 applications** | |
| **Step 1** | **Step 2** | **Step 1** | **Step 2** |
| **EED (CFU/L)** | **6.00E+06** | **4.44E+05** | **3.50E+07** | **2.60E+06** |
| **PEC (ITU/L)** | **1.00E+03** | **7.41E+01** | **3.20E+03** | **2.37E+02** |

Acceptable risks occurs when the predicted density and biopotency are calculated considering adsorption on sediment, and further refinements considering degradation over several days, as presented in the Annex 1 CAR were therefore not reported here.

For sediment compartment, some mistakes should have occured in the Annex 1 CAR for the calculation of the predicted density and biopotency in the case of multiple applications. At first, values reported in the CAR correspond to the following equations :

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

and

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

Without taking into account the following equations :

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

and

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

and

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

and

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

However results between the two calculations remain similar (see the table below) and as no PNED and PNEC are available for the sediment, no risk assessment has been performed.

Table 20: predicted *Bti* AM65-52 density and biopotency in sediment after ground application of 1kg/ha of VECTOBAC WG at surface water

|  |  |  |  |
| --- | --- | --- | --- |
|  | **1 application (step 2)** | **8 applications (step 2 – CAR values)** | **8 applications (step 2- re-assessed values)** |
| **EED (CFU/g)** | **2.22E+04** | **1.30E+05** | **1.41E+05** |
| **PEC (ITU/g)** | **3.7** | **1.19E+01** | **1.52E+01** |

**STP**

Applications in waste water, sewage effluent and lagoons are intended and exposure from this use is therefore briefly presented. For these uses, it is considered that ‘VECTOBAC’ WG is applied at the same rate as it is applied to other water bodies, with a maximum of 1 kg/ha. As the application rate is the same, it can reasonably be expected that the concentration of *Bti* in the sewage treatment plant effluent will not exceed the values calculated for other water bodies which are reported in the table below

Table 21: predicted *Bti* AM65-52 density and biopotency in STP after ground application of 1kg/ha of VECTOBAC WG

|  |  |  |
| --- | --- | --- |
|  | **1 application (step 2)** | **8 applications (step 2)** |
| **EED (CFU/L)** | **4.44E+05** | **3.50E+07** |
| **PEC (ITU/L)** | **7.41E+01** | **3.20E+03** |

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 7 days between applications. In the Annex 1 CAR, it was assumed that VECTOBAC WG was applied directly to soil at the maximum application rate of 1 kg/ha without taking any drift factor into account. However in the Annex I CAR, a conversion mistake has been detected and corrections lead to unacceptable risks for the soil compartment. Therefore, the exposure to soil was re-calculated and 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 re-calculated and refined from the maximum application rate to water (1kg/ha) and using an appropriate drift factor from Rautmann et al. (1999)[[5]](#footnote-5). 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. For the applicant, as it is expected that the aim will be to apply the product to the centre of the water bodies to allow outward homogenisation, a distance of 15 m seems to represent a conservative estimate of the distance between the application site and any adjacent non target soil areas such as fields or grassland. Based on this information a drift value of 4.21% was chosen by the applicant. Nevertheless, the RMS prefer to choose 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\*7)

and

PECsoil, final, n = PECsoil, init, n xe(-ln(2)/DT50\*7)

With the following symbols:

EEDsoil, t=0: *Bti* AM65-52 initial population density in soil before any dissipation (CFU/kg)

EEDsoil, init, n: *Bti* AM65-52 population density in soil following n applications (CFU/kg)

EEDsoil, final, n: *Bti* AM65-52 population density in soil after 7 days following n applications (CFU/kg)

PECsoil, t=0: *Bti* AM65-52 initial biopotency in soil before any dissipation (ITU/kg)

PECsoil, init, n: *Bti* AM65-52 biopotency in soil following n applications (ITU/kg)

PECsoil, final, n: *Bti* AM65-52 biopotency in soil after 7 days following n applications (ITU/kg)

Results are reported in the table below:

Table 22: predicted *Bti* AM65-52 density and biopotency in soil after gound application of 1kg/ha of VECTOBAC WG at surface water

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **1 application (drift = 100%)** | **8 applications (drift = 100%)** | **1 application (drift = 22.24%)** | **8 applications**  **(drift = 22.24%)** |
| **EED (CFU/kg)** | **2.40E+07** | **1.67E+08** | **5.34E+06** | **3.72E+07** |
| **PEC (ITU/kg)** | **4.00E+03** | **6.59E+03** | **8.9E+02** | **1.47E+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 AM65-52 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

##### 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 2.4.8.2.2.

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

For the aerial application, no drift value is proposed by the applicant and 100% of application of VECTOBAC WG is therefore considered, as a worst case. Additionally, the exposure to soil was calculated using a drift factor according to FOCUS 2011[[6]](#footnote-6). 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 23: predicted *Bti* AM65-52 density and biopotency in soil after aerial application of 1kg/ha of VECTOBAC WG in surface water

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **1 application** | **2 applications** | **3 applications** | **8 applications** |
| **Drift = 100%** | | | | |
| EED (CFU/kg) | 2.40E+07 | 4.70E+07 | 6.92E+07 | 1.67E+08 |
| PEC (ITU/kg) | 4.00E+03 | 5.57E+03 | 6.19E+03 | 6.59E+03 |
| **Drift = 27.3%** | | | | |
| EED (CFU/kg) | 6.55E+06 | 1.28E+07 | 1.89E+07 | 4.57E+07 |
| PEC (ITU/kg) | 1.09E+03 | 1.52E+03 | 1.69E+03 | 1.80E+03 |

**Groundwater**

Same assumptions than for ground application occur. See 2.4.8.2.3

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

Same assumptions than for ground application occur. See 2.4.8.2.4

### 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

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

Table 24: PEC/PNEC ratios for different exposure situations concerning the surface water, considering ground application of 1kg/ha of VECTOBAC WG at surface water

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Exposure scenario** | **EED - PEC** | **EED/PNED - PEC/PNEC** | | **Risks** | |
|  | Water/local (PNEDwater = 1 x107 CFU/L  PNECwater = 3.3x102 ITU/L) | | | | |
| Density | 8 applications (Step 1)  EED = 3.50E+07 CFU/L | | 3.50 | | Not acceptable |
| 8 applications (Step 2)  EED = 2.60E+06 CFU/L | | 0.26 | | Acceptable |
| Biopotency | 8 applications (Step 1)  PEC = 3.20E+03 ITU/L | | 9.37 | | Not acceptable |
| 8 applications (Step 2)  PEC = 2.37E+02 ITU/L | | 0.72 | | Acceptable |

##### When adsorption sediment is taken into account (Step 2), the EED/PNED and PEC/PNEC ratio are below 1 for aquatic compartment indicating acceptable risk. However, risk assessment based on biopotency has been carried out taking into account the nominal biopotency of 3000 ITU/mg. No maximal potency is available and it should be noted that unacceptable risks would be reached for a biopotency of 4167 ITU/mg. RMS considers that biopotency is a good parameter to assess the environmental risk of the toxin contained in Bti, nevertheless there is at present no clear consensus about this issue.

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 2.4.8.2.2.

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

Table 25: PEC/PNEC ratios for different exposure situations concerning the soil after ground application of 1kg/ha of VECTOBAC WG at surface water

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Exposure scenario** | **EED - PEC** | **EED/PNED - PEC/PNEC** | | **Risks** | |
|  | Water/local (PNEDsoil = 4.8 x107 CFU/kg  PNECsoil = 8.0 x103 ITU/kg) | | | | |
| Density | 8 applications (Drift 100%)  EED = 1.67E+08 CFU/L | | 3.48 | | Not acceptable |
| 8 applications (Drift 22.24%)  EED = 3.72E+07 CFU/L | | 0.77 | | Acceptable |
| Biopotency | 8 applications (Drift 100%))  PEC = 6.59E+03 ITU/L | | 0.82 | | Acceptable |
| 8 applications (Drift 22.24%)  PEC = 1.47E+03 ITU/L | | 0.18 | | Acceptable |

Based on predicted biopotency, ground application of 1kg/ha of VECTOBAC WG lead to acceptable risk for the soil, whatever the drift value considered. Based on predicted density, EED/PNED is below 1 only when the refined value of drift is considered and indicate acceptable risk for soil compartment in the case of ground application.

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

See 2.4.8.2.4

##### Conclusions

Eight ground applications of VECTOBAC WG at 1 kg/ha, with an interval of at least 7 days between two applications, lead to acceptable risk for the environment. However, the maximal biopotency of the product, which is at present not determined, should not be above 4167 ITU/mg.

Nevertheless, as no toxicity data are provided for sediment organisms which was accepted at the EU level for the inclusion of the substance and as contradictory results with some predator of targeted organisms are reported in the litterature, effects arising from long term and large scale use of the product on natural biological diversity should be assessed. Appropriate mitigation measures should be adapted in the case of potential identified risks.

#### Intended use 2: aerial application

##### 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 2.8.5.1 for this risk characterisation.

##### Atmospheric compartment

See 2.4.8.2.2.

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

Table 26: PEC/PNEC ratios for different exposure situations concerning the soil after aerial application of 1 kg/ha of VECTOBAC WG at surface water

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Exposure scenario** | **EED - PEC** | **EED/PNED - PEC/PNEC** | | **Risks** | |
|  | Water/local (PNEDsoil = 4.8 x107 CFU/kg  PNECsoil = 8.0 x103 ITU/kg) | | | | |
| **Drift = 100%** | | | | | |
| Density | 1 application  EED = 2.40E+07 CFU/kg | | 0.5 | | Acceptable |
| 2 applications  EED = 4.70E+07 CFU/kg | | 0.98 | | Acceptable |
| 3 applications  EED = 6.92E+07 CFU/kg | | 1.44 | | **Not acceptable** |
| 8 applications  EED = 1.67E+08 CFU/kg | | 3.48 | | **Not acceptable** |
| Biopotency | 1 application  PEC = 4.00E+03 ITU/kg | | 0.5 | | Acceptable |
| 2 applications  PEC = 5.57E+03 ITU/kg | | 0.70 | | Acceptable |
| 3 applications  PEC = 6.19E+03 ITU/kg | | 0.77 | | Acceptable |
| 8 applications  PEC = 6.59E+03 ITU/kg | | 0.82 | | Acceptable |
| **Drift = 27.3%** | | | | | |
| Density | 1 application  EED = 6.55E+06 CFU/kg | | 0.14 | | Acceptable |
| 8 applications  EED = 4.57E+07 CFU/kg | | 0.95 | | Acceptable |
| Biopotency | 1 application  PEC = 1.09E+03 ITU/kg | | 0.14 | | Acceptable |
| 8 applications  PEC = 1.80E+03 ITU/kg | | 0.225 | | Acceptable |

Based on biopotency and predicted density, PEC/PNEC and EED/PNED are below 1 which indicates acceptable risk for soil compartment in the case of aerial application.

For the aerial application, no drift value is proposed by the applicant and 100% of application of VECTOBAC WG is therefore considered, as a worst case.

In this case, acceptable risks are predicted for 1 and 2 applications and for the highest intended dose of 1kg /ha.  However EED/PNED would be over 1, for 3 or more aerial applications.

The applicant claimed until max. 8 applications. Nonetheless, in practice, the number of aerial applications and the interval between each application depends on targeted species, infestation level and climatic conditions.

Nevertheless, taking into account the aerial drift values proposed in the FOCUS Surface Water Scenarios in the EU Evaluation Process under 91/414/EEC[[7]](#footnote-7)[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.

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.

Besides, 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 there is no mechanism for the ingestion of *Bti* AM65-52 and therefore no appropriate digestive enzymes to enable the release of the active protein δ-endotoxins. Additionally, the PNED soil value (4.8x107 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, in the case of repeated aerial applications on large scale, 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.

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

See 2.4.8.2.4

##### Conclusions

Eight aerial applications of VECTOBAC WG at 1 kg/ha, with an interval of at least 7 days between two applications, lead to acceptable risk for the aquatic compartment. However, the maximal biopotency of the product, which is at present not determined, should not be above 4167 ITU/mg.

Nevertheless, as no toxicity data are provided for sediment organisms and as contradictory results with some predator of targetted organisms are reported in the litterature, effects arising from long term and large scale use of the product on natural biological diversity should be assessed. Appropriate mitigation measures should be adapted in the case of potential identified risks.

Acceptable risks for the terrestrial compartment are predicted for 1 and 2 aerial applications and for the highest intended dose of 1kg /ha.  However EED/PNED would be over 1, for 3 or more aerial applications. However more aerial applications could be required and refinement of the assessment, as reducing drift value taking into account a non treated area appears as not relevant for an efficient treatment against mosquitos. Nevertheless, it should be kept in mind that the PNED soil value is in the same order of magnitude that the density of *Bacillus thuringiensis* that occurs in soil. Additionally, the PNED soil value is derived from an acute earthworm study showing no adverse effect at the highest tested concentration and adverse effect are neither expected for soil microorganisms and terrestrial plants. Therefore, in the case of repeated aerial applications on large scale, 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***

* For ground uses, do not exceed 8 applications with an interval of at least 7 days between two applications.
* Aerial application is only allowed when ground application is not feasible.
* Aerial application is only allowed for areas larger than 0.5 ha.
* The person responsible for the control shall ensure that the application equipment is suitable for the type of aircraft, calibrated properly and that wind drift is minimized at the application site, in order to ensure correct dosage and avoid exposure to soil.
* The aircraft should be equipped with a professional GPS Guidance system enabling precise application of VectoBac WG where granted.
* 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 VectoBac WGin a natural water habitat.
* When applying VectoBac WG 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.

***Required information linked to risk assessment for environment.***

* Effects arising from long term and large scale uses of the product on natural biological diversity including aquatic food webs should be reported by the authorization holder to Competent Authorities (CA).
* From 3 aerial applications on large scale, effect of these applications on natural biological diversity including terrestrial food webs should be reported by the authorization holder to Competent Authorities (CA),
* Data dealing with the assessment of effect on biodiversity should be provided every 24 months

## Measures to protect man, animals and the environment

*See Summary of Product Characteristics (SPC)*

# Proposal for decision – Post Authorisation – 2021 :

**Conclusions of efficacy and risk assessment**

***Risk assessment for Physico-chemical properties***

VECTOBAC WG is Wettable Granule (WG). It is not highly flammable, not auto-flammable at ambient temperature, not explosive and does not have oxidizing properties.

The product is stable two years at ambient temperature. Therefore the shelf life of the product is 2 years. The product VECTOBAC WG is compatible with HDPE.

The formulation must not be stored at a temperature above 25 °C.

The formulation should be protecting from the light.

***Summary of efficacy assessment***

The efficacy level of the product VECTOBAC WG (37% w/w *Bti* AM 65-52) is satisfactory for the uses proposed in annex0b.

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

No unacceptable risk has been identified for professionals using VECTOBAC WG with ground or aerial equipment when appropriate PPE were worn.

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 working coverall and gloves are worn in case of re-entry after treatment on rice.

***Summary of risks characterisation of the product for consumer***

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. VECTOBAC WG should not be applied in waters in irrigated crops, except in water surrounding rice for which a pre harvest interval of 1 month is required.

***Summary of risks characterisation of the product for the environment***

Eight ground or aerial application of VECTOBAC WG at 1 kg/ha with an interval of at least 7 days between two applications, lead to acceptable risk for the aquatic compartment, assuming a maximal biopotency of 4167 ITU/mg.

Nevertheless, as no toxicity data are provided for sediment organisms and as contradictory results with some predator of targetted organisms are reported in the litterature, effects arising from long term and large scale use of the product on natural biological diversity should be assessed. Appropriate mitigation measures should be adapted in the case of potential identified risks.

Acceptable risks are also predicted for the soil compartment in the case of ground applications. However in the case of aerial applications, acceptable risks are only identified for 1 and 2 aerial applications and for the highest intended dose of 1kg/ha.  For higher number of applications, it appears difficult to refine the exposure assessment taking into account mitigation measures which could reduce the efficiency of the treatement against mosquitoes (such as non treated area). Nevertheless, the PNED soil value is in the same order of magnitude that the density of Bacillus thuringiensis that occurs in soil and is moreover derived from studies showing no adverse effect at the highest tested concentrations. Therefore, in the case of repeated aerial applications on large scale, 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 and conditions of use**

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

* Do not store at temperatures higher than 4°C.
* Protect from the light.
* Shelf life: 2 years
* Stir the diluted product before and during the application

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

To ensure a satisfactory level of efficacy, 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.

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

Professionals must wear gloves, working coverall, goggles and respiratory mask (with P3 filter)

* VECTOBAC WG 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 users are not permitted in area being treated.
* A drift buffer zone of 50 m should be respected after aerial application and when the product is applied with a vehicle mounted motorized spray equipment. Another mode of application (such as with a portable sprayer) should be used in areas closed to housing.
* In case of re-entry after treatment on rice, it is recommended to workers to wear a working coverall and gloves.
* VECTOBAC WG should not be applied in water surrounding crops, except in water surrounding rice for which a pre harvest interval of 1 month is required.

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

* do not be applied in waters in irrigated crops, except in water surrounding rice for which a pre harvest interval of 1 month is required

***Emergency*** *(information provided in the product Safety Data Sheet)*

* Not evaluated by Anses.
* Wear suitable protective clothing during handling the product. Avoid contact with skin, eyes and respiratory tract. During treatment, wear suitable protective clothing. Do not eat, drink or smoke during application and until your hands have been washed.
* Advice to doctor: symptomatic treatment is advised.

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

* For ground uses, do not exceed 8 applications with an interval of at least 7 days between two applications
* Aerial application is only allowed when ground application is not feasible.
* Aerial application is only allowed for areas larger than 0.5 ha.
* The person responsible for the control shall ensure that the application equipment is suitable for the type of aircraft, calibrated properly and that wind drift is minimized at the application site, in order to ensure correct dosage and avoid exposure to soil.
* The aircraft should be equipped with a professional GPS Guidance system enabling precise application of VectoBac WG where granted.
* 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 VectoBac WGin a natural water habitat.
* When applying VectoBac WG 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.

**Information required post-authorisation**

***Required information linked to assessment of physico-chemical properties and analytical methods***

* A maximum biopotency of the product should be set and is required in post registration.
* The formulation must not be stored at a temperature above 25 °C during 24 months.
* The formulation should be protecting from the light.
* Data on the determination of microbial contaminants indicated in OECD 65 “issue paper on microbial contaminant limits for microbial pest control products (Oct. 2011)”and the persistent foaming according to GIFAP n° 17 are required before and after storage of the product at 25°C for 2 years in the commercial package (HDPE) are required in post registration.
* The dispersibility should be performed at the maximum use concentration (40 %w/v) and is required in post registration.
* The composition detail of other claimed package fiber drum should be provided in post registration.
* The determination of *Salmonella and yeast and mould*  in five batches of the formulation VECTOBAC WG remains missing and is required according to OECD 65 are required in post registration. This determination should be performed with validated methods (at least positive controls and repeatability) or with international standard methods which are required in post registration.
* *Staphylococcus aureus* should be determined in post registration in five batches with a validated method with a detection limit < 10 cfu/g according to OECD 65 (absence in 1g).
* **Post-authorisation data – 2018 :**

The maximum biopotency of the product (3800 ITU/mg) was provided and considered **acceptable**

The determination of the *Salmonella* and *Staphylococcus aureus* in five batches of the product Vectobac WG using a validated method with a limit of detection of 10 CFU/g instead of **“**absent in 25 g” and “absent in 1 g” respectively was provided and acceptable. **Nevertheless, the confirmation of absence in 25 g for *Salmonella* and in 1 g for *Staphylococcus aureus* was missing and was required**.

The detail of the specifications of packaging “Fiber drum” was provided and considered acceptable.

Every previous post-authorisation data set at the first authorisation and not provided or acceptable are still required.

* **Post-authorisation data – 2020 :**

**The following data were provided and considred acceptable:**

* an acceptable justification for the spontaneity of dispersion at the maximum use concentration (40 %(w/v)), The product should be stired before and during application.

**The following data were not provided but the gap can be considred acceptable:**

* the determination of the *Salmonella* and *Staphylococcus aureus* in five batches of the product Vectobac WG using a validated method with the criteria of absence in 25 g and absence in 1 g respectively according to the document OECD 65 (oct. 2011), for confirmation. As acceptable results in 10 g of five batches of Vectobac WG are available, a confirmatory data for the determination of *Salmonella* and *Staphylococcus aureus* in 25 and 1 g of the product respectively are not necessary.
* the determination of microbial contaminants according to the document OECD 65 (oct. 2011) in the same batch of the product Vectobac WG before and after 24 months at 4 °C. The results are in the acceptable limits **but cannot be extrapolated at 25 °C. Considering the absence of the results at 25 °C the mitigation measure should be changed to: “Do not store at temperature above 4 °C” instead of “Do not store at temperature above 25°C”**

**The following data were not provided and remain missing (Not acceptable, data gap):**

* the determination of yeast and mould in five batches of the product Vectobac WG using validated methods or international standard methods according to OECD 65 (Oct. 2011).
* a study of the persistent foaming before and afer 24 months at 25 °C in the same batch of the product according to FAO manual (2010) and according to the GIFAP n° 17 (2009).

It should be noted that a stability study plan (24 months at 25 °C) for the determination physical and chemical properties on the product VBC-60782 (100% Bti AM 65-52) was provided **Nevertheless, as the product is different from Vectobac WG, the results of persistent foaming on VBC-60782 could not be used to conclude on Vectobac WG**. **Furthermore, the study plan does not include the determination of microbial contaminant at 25 °C.**

***Required information linked to efficacy assessment***

* The authorization holder has to report any observed resistance incidents to the Competent Authorities (CA) or other appointed bodies involved in resistance management.

***Required information linked to assessment of the environment***

* Effects arising from long term and large scale use of the product on natural biological diversity including aquatic food webs should be reported by the authorization holder to Competent Authorities (CA).
* In the case of more than 2 aerial applications on large scale, effect of these applications on natural biological diversity including terrestrial food webs should be reported by the authorization holder to Competent Authorities (CA),
* Data dealing with the assessment of effect on biodiversity should be provided every 24 months.

# Appendices

# Annex 0a: Practical use claimed by the applicant for VECTOBAC WG

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Name of the product and type of formulation (gel, paste, spray, dust, powder, fumigation…)** | **Target organisms (common species and genus) and development stages (eggs, larvae, nymph, adults…)\*** | **User category (professional/non professional)\*** | **Application aim** | **Area of use (indoor, outdoor, and field of use )** | **Method of application** | **Application rate (expressed in g/m3, g/m2, ml/m2…)**  **Maximum and minimum dosage (if appropriate)** | **Mode of action including time delay (kill, knockdown...)** | **Time delay of residual efficacy if indirect or surface treatment ( hours, days, weeks and months)** | **Time delay for human , food and animals reentrance after treatment (if appropriate)** | **Frequency and duration of application** | **Dosage and applications requirements (exposure time, ventilation, temperature,)** | **Package details : Individual packaging (yes/no)\*\*** | **Primary packaging \*\*\* : type : bulk, individual wrapping…/ nature: bucket, bottle, sachet…/ material: paper, polyethylene…/ sizes** | **Secondary packaging** | **Accepted and authorized by the RMS (yes/no)** |
| **NAME**  Formulation: VECTOBAC WG | Larval stages L1-early L4 of house mosquitoes such as *Culex spp.* and *Culistea spp.*; *Anopheles spp*.; and floodwater mosquitoes *Aedes spp*. and *Ochlerotatus spp*. | Professional | Control of mosquitoes | Outdoor: Relatively clear water in which mosquito larvae proliferate such as irrigation ditches; reservoirs, lakes, rivers, river flood plains, rice field, canals, marshland, ponds; catch basins, drainage and roadside ditches; all other natural or manmade aquatic sites or containers in which mosquito larvae are actively developing. | Portable pump pressure sprayer; Motorized portable blower Motorized portable blower | 0.125-0.5 kg/ha in 2.5-500 l water/ha | The larvae must ingest the product. The insecticidal activity occurs in the insect gut. Thereafter they will die, thus not developing into adult mosquitoes. | The product is effective as soon as the target species ingest the insecticidal crystal protein. | Immediately after the application; there are no concern with respect to safety to humans and animals | 1 application per generation of target species. Each time new larval populations are present. Up to max. 8 applications per season. | The product is effective as soon as the target species ingest the insecticidal crystal protein. | Yes | 0.5, 5.0 kg HDPE container, or 25 kg fiber drums\* | None |  |
| Larval stages L1-early L4 of house mosquitoes such as *Culex spp.* and *Culistea spp.*; *Anopheles spp*.; and floodwater mosquitoes *Aedes spp*. and *Ochlerotatus spp*. | Professional | Control of mosquitoes | Outdoor: Relatively clear water in which mosquito larvae proliferate such as irrigation ditches; reservoirs, lakes, rivers, river flood plains, rice field, canals, marshland, ponds; catch basins, drainage and roadside ditches; all other natural or manmade aquatic sites or containers in which mosquito larvae are actively developing. | Aerial spray, with low drift flat fan nozzles or air inductor nozzles; Droplet size is usually between 400-800 µm are considered coarse drops, which minimises drift | 0.125-0.5 kg/ha in 2.5-500 l water/ha | The larvae must ingest the product. The insecticidal activity occurs in the insect gut. Thereafter they will die, thus not developing into adult mosquitoes. | The product is effective as soon as the target species ingest the insecticidal crystal protein. | Immediately after the application; there are no concern with respect to safety to humans and animals | 1 application per generation of target species. Each time new larval populations are present. Up to max. 8 applications per season. | The product is effective as soon as the target species ingest the insecticidal crystal protein. | Yes | 0.5, 5.0 kg HDPE container, or 25 kg fiber drums\* | None |  |
| Larval stages L1-early L4 of house mosquitoes such as *Culex spp.* and *Culistea spp.*; *Anopheles spp*.; and floodwater mosquitoes *Aedes spp*. and *Ochlerotatus spp*. | Professional | Control of mosquitoes | Outdoor: Relatively clear water in which mosquito larvae proliferate such as irrigation ditches; reservoirs, lakes, rivers, river flood plains, rice field, canals, marshland, ponds; catch basins, drainage and roadside ditches; all other natural or manmade aquatic sites or containers in which mosquito larvae are actively developing. | Vehicle mounted motorized spray equipment | 0.125-0.5 kg/ha in 2.5-500 l water/ha | The larvae must ingest the product. The insecticidal activity occurs in the insect gut. Thereafter they will die, thus not developing into adult mosquitoes. | The product is effective as soon as the target species ingest the insecticidal crystal protein. | Immediately after the application; there are no concern with respect to safety to humans and animals | 1 application per generation of target species. Each time new larval populations are present. Up to max. 8 applications per season. | The product is effective as soon as the target species ingest the insecticidal crystal protein. | Yes | 0.5, 5.0 kg HDPE container, or 25 kg fibre drums\* | None |  |
| Larval stages L1-early L4 of house mosquitoes such as *Culex spp.* and *Culistea spp.*; *Anopheles spp*.; and floodwater mosquitoes *Aedes spp*. and *Ochlerotatus spp*. | Professional | Control of mosquitoes | Outdoor: Relatively clear water in which mosquito larvae proliferate such as irrigation ditches; reservoirs, lakes, rivers, river flood plains, rice field, canals, marshland, ponds; catch basins, drainage and roadside ditches; all other natural or manmade aquatic sites or containers in which mosquito larvae are actively developing. | Aerial application of product frozen as ice granules, with appropriate granule spreader. Ice granule size: 2-8 mm | 0.125-0.5 kg/ha in 5-30 litres of water frozen to ice granules | The larvae must ingest the product. The insecticidal activity occurs in the insect gut. Thereafter they will die, thus not developing into adult mosquitoes. | The product is effective as soon as the target species ingest the insecticidal crystal protein. | Immediately after the application; there are no concern with respect to safety to humans and animals | 1 application per generation of target species. Each time new larval populations are present. Up to max. 8 applications per season. | The product is effective as soon as the target species ingest the insecticidal crystal protein. | Yes | 0.5, 5.0 kg HDPE container, or 25 kg fibre drums\* | None |  |
| Larval stages L1-early L4 low and high infestation homogeneous and heterogeneous population or were L4 larvae are predominant; Target mosquitoes house mosquitoes such as *Culex spp.* and *Culistea spp.*; *Anopheles spp*.; and floodwater mosquitoes *Aedes spp*. and *Ochlerotatus spp*. | Professional | Control of mosquitoes | Outdoor: Relatively dirty, polluted water, or containing high levels of organic matter in which mosquito larvae proliferate such as rice fields, river flood plains, wastewater; sewage effluent and lagoons, septic ditches; animal waste lagoons; All other natural or manmade aquatic sites or containers, whether the water is clean or dirty, in which mosquito larvae are actively developing were a higher dose is required to get sufficient mortality: For example at low water temperatures, high larval density and predominance of late L4 stages | Portable pump pressure sprayer; Motorized portable blower Motorized portable blower | 0.5-1.0 kg/ha in 2.5-500 l water/ha | The larvae must ingest the product. The insecticidal activity occurs in the insect gut. Thereafter they will die, thus not developing into adult mosquitoes. | The product is effective as soon as the target species ingest the insecticidal crystal protein. | Immediately after the application; there are no concern with respect to safety to humans and animals | 1 application per generation of target species. Each time new larval populations are present. Up to max. 8 applications per season. | The product is effective as soon as the target species ingest the insecticidal crystal protein. | Yes | 0.5, 5.0 kg HDPE container, or 25 kg fibre drums\* | None |  |
| Larval stages L1-early L4 low and high infestation homogeneous and heterogeneous population or were L4 larvae are predominant; Target mosquitoes house mosquitoes such as *Culex spp.* and *Culistea spp.*; *Anopheles spp*.; and floodwater mosquitoes *Aedes spp*. and *Ochlerotatus spp*. | Professional | Control of mosquitoes | Outdoor: Relatively dirty, polluted water, or containing high levels of organic matter in which mosquito larvae proliferate such as rice fields, river flood plains, wastewater; sewage effluent and lagoons, septic ditches; animal waste lagoons; All other natural or manmade aquatic sites or containers, whether the water is clean or dirty, in which mosquito larvae are actively developing were a higher dose is required to get sufficient mortality: For example at low water temperatures, high larval density and predominance of late L4 stages | Aerial spray, with low drift flat fan nozzles or air inductor nozzles; Droplet size is usually between 400-800 µm are considered coarse drops, which minimises drift | 0.5-1.0 kg/ha in 2.5-500 l water/ha | The larvae must ingest the product. The insecticidal activity occurs in the insect gut. Thereafter they will die, thus not developing into adult mosquitoes. | The product is effective as soon as the target species ingest the insecticidal crystal protein. | Immediately after the application; there are no concern with respect to safety to humans and animals | 1 application per generation of target species. Each time new larval populations are present. Up to max. 8 applications per season. | The product is effective as soon as the target species ingest the insecticidal crystal protein. | Yes | 0.5, 5.0 kg HDPE container, or 25 kg fibre drums\* | None |  |
| Larval stages L1-early L4 low and high infestation homogeneous and heterogeneous population or were L4 larvae are predominant; Target mosquitoes house mosquitoes such as *Culex spp.* and *Culistea spp.*; *Anopheles spp*.; and floodwater mosquitoes *Aedes spp*. and *Ochlerotatus spp*. | Professional | Control of mosquitoes | Outdoor: Relatively dirty, polluted water, or containing high levels of organic matter in which mosquito larvae proliferate such as rice fields, river flood plains, wastewater; sewage effluent and lagoons, septic ditches; animal waste lagoons; All other natural or manmade aquatic sites or containers, whether the water is clean or dirty, in which mosquito larvae are actively developing were a higher dose is required to get sufficient mortality: For example at low water temperatures, high larval density and predominance of late L4 stages | Vehicle mounted motorized spray equipment | 0. 5-1.0 kg/ha in 2.5-1000 l water/ha | The larvae must ingest the product. The insecticidal activity occurs in the insect gut. Thereafter they will die, thus not developing into adult mosquitoes. | The product is effective as soon as the target species ingest the insecticidal crystal protein. | Immediately after the application; there are no concern with respect to safety to humans and animals | 1 application per generation of target species. Each time new larval populations are present. Up to max. 8 applications per season. | The product is effective as soon as the target species ingest the insecticidal crystal protein. | Yes | 0.5, 5.0 kg HDPE container, or 25 kg fibre drums\* | None |  |
| Larval stages L1-early L4 low and high infestation homogeneous and heterogeneous population or were L4 larvae are predominant; Target mosquitoes house mosquitoes such as *Culex spp.* and *Culistea spp.*; *Anopheles spp*.; and floodwater mosquitoes *Aedes spp*. and *Ochlerotatus spp*. | Professional | Control of mosquitoes | Outdoor: Relatively dirty, polluted water, or containing high levels of organic matter in which mosquito larvae proliferate such as rice fields, river flood plains, wastewater; sewage effluent and lagoons, septic ditches; animal waste lagoons; All other natural or manmade aquatic sites or containers, whether the water is clean or dirty, in which mosquito larvae are actively developing were a higher dose is required to get sufficient mortality: For example at low water temperatures, high larval density and predominance of late L4 stages | Aerial application of product frozen as ice granules, with appropriate granule spreader. Ice granule size: 2-8 mm | 0.5-1.0 kg/ha in 5-30 litres of water frozen to ice granules | The larvae must ingest the product. The insecticidal activity occurs in the insect gut. Thereafter they will die, thus not developing into adult mosquitoes. | The product is effective as soon as the target species ingest the insecticidal crystal protein. | Immediately after the application; there are no concern with respect to safety to humans and animals | 1 application per generation of target species. Each time new larval populations are present. Up to max. 8 applications per season. | The product is effective as soon as the target species ingest the insecticidal crystal protein. | Yes | 0.5, 5.0 kg HDPE container, or 25 kg fibre drums\* | None |  |

* **Post authorisation data – 2018 :**

\*Packaging specifications of fiber drums (VALENT BIOSCIENCES CORPORATION COMMODITY SPECIFICATION DOCUMENT CN.76.0162 (Packaging\_drum.pdf)):

|  |  |
| --- | --- |
| MATERIAL/CHARACTERISTICS: |  |
| COLOR | INSIDE:  KRAFT  OUTSIDE: PMS 282 BLUE OR PMS 289 BLUE |
| TOP/LID | METAL, 24 GAUGE STEEL (COATED 2 SIDES.  0.2 FOOD GRADE EPOXY PHENOLIC). |
| BODY | N/A |
| SIDEWALL | FIBER PLIES CONVOLUTELY WOUND, CONSISTING OF .012" MINIMUM THICKNESS. ALL SIDEWALL PLIES JOINED WITH SODIUM SILICATE. OUTER PLY TO BE BLUE POLY/KRAFT LAMINATE WITH BLACK TAPE SEAM; OVERWRAP TO BE  .00125" GLOSS BLUE POLY LAMINATED TO APPROXIMATELY 46 POUND KRAFT PAPER. |

# Annex 0b: Proposed uses for the authorization of VECTOBAC WG

|  |  |  |  |
| --- | --- | --- | --- |
| **Target Organismes** | **Rates and uses acceptable** | **Method of application** | **Recommandations** |
| Mosquito  *genus Culex, Culiseta*  *genus Aedes* (*Ochlerotatus*)  *genus Anopheles*  from 1st larval stage to earlier 4th stage | **Low infestation**  0.125 – 0.5 kg/ha  **High infestation**  0.5-1 kg/ha  The product must be dispersed in water prior to application. The volume of water varies between 2.5 and 500 L  The dose rates may be depend on the population density and water quality. The lowest dose rates provide adequate control of 1st through early of 4th instar larvae. in cases of predominance of 4th instar larvae, high population densities, water containing high levels of organic matter, colder temperatrure, and / or significant water exchange, higher rates should be used to provide good control of mosquitoes. | **Terrestrial and aerial application**  VECTOBAC WG si applied by  portable pump pressure sprayer, motorized portable blower equipment) by airplane equipement). | VECTOBAC WG is applied by professionnals in larvae breeding sites  The maximum number of application is 8 with 7 days minimum interval between each application. |
|  | **Low infestation**  0.125 – 0.5 kg/ha  **High infestation**  0.5 -1 kg/ha  The product must be dispersed in water prior to application. The volume of water varies between 5 and 30 L  The dose rates may be depend on the population density and water quality. The lowest dose rates provide adequate control of 1st through early of 4th instar larvae. in cases of predominance of 4th instar larvae, high population densities, water containing high levels of organic matter, colder temperatrure, and / or significant water exchange, higher rates should be used to provide good control of mosquitoes. | **Aerial application**  VECTOBAC WG is applied by a spreader in helicopter for ice granules |
| **Low infestation**  0.125 – 0.5 kg/ha  The product must be dispersed in water prior to application. The volume of water varies between 2.5 and 500 L  **High infestation**  0.5-1 kg/ha  The product must be dispersed in water prior to application. The volume of water varies between 2.5 and 1000 L.  The dose rates may be depend on the population density and water quality. The lowest dose rates provide adequate control of 1st through early of 4th instar larvae. in cases of predominance of 4th instar larvae, high population densities, water containing high levels of organic matter, colder temperatrure, and / or significant water exchange, higher rates should be used to provide good control of mosquitoes. | **Terrestrial application**  VECTOBAC WG is applied by vehicle mounted motorized sprayer equipment |

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Annex 1: Summary of product characteristics

*See separated file.*

Annex 2: 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** | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Section 1** | | | | | | **Y** | **No** |
| No study reports submitted | | | | | |
| **Section 2** | | | | | |
| IIIB, 2.1/01 | Young, S. | 2003 | ‘VECTOBAC’ WG two year storage stability. Huntingdon Life Sciences Ltd., report no. ABT 401/024588. GLP, unpublished. | Y | Valent Biosciences |  |  |
| IIIB, 2.2.1/01 | Young, S. | 2003 | ‘VECTOBAC’ WG two year storage stability. Huntingdon Life Sciences Ltd., report no. ABT 401/024588. GLP, unpublished. | Y | Valent Biosciences |  |  |
| IIIB, 2.3/01 Confidential | Curl, M.G. | 2005a | Expert statement on the explosive properties of ‘VECTOBAC’ WG formulated preparation. TSGE report no. 22-1-05.EXP. Non-GLP, unpublished | Y | Valent Biosciences |  |  |
| IIIB, 2.3/02 Confidential | Curl, M.G. | 2005b | Expert statement on the oxidising properties of ‘VECTOBAC’ WG formulated preparation. TSGE report no. 22-1-05.OXP. Non-GLP, unpublished | Y | Valent Biosciences |  |  |
| IIIB, 2.4/01 Confidential | Curl, M.G. | 2005c | Expert statement on the flammability of ‘VECTOBAC’ WG formulated preparation. TSGE report no. 22-1-05.FLM. Non-GLP, unpublished | Y | Valent Biosciences |  |  |
| IIIB, 2.5/01 | Young, S. | 2003 | ‘VECTOBAC’ WG two year storage stability. Huntingdon Life Sciences Ltd., report no. ABT 401/024588. GLP, unpublished. | Y | Valent Biosciences |  |  |
| IIIB, 2.7.1/01 | Young, S. | 2003 | ‘VECTOBAC’ WG two year storage stability. Huntingdon Life Sciences Ltd., report no. ABT 401/024588. GLP, unpublished. | Y | Valent Biosciences |  |  |
| IIIB, 2.7.2/01 | Young, S. | 2003 | ‘VECTOBAC’ WG two year storage stability. Huntingdon Life Sciences Ltd., report no. ABT 401/024588. GLP, unpublished. | Y | Valent Biosciences |  |  |
| IIIB, 2.7.3/01 | Young, S. | 2003 | ‘VECTOBAC’ WG two year storage stability. Huntingdon Life Sciences Ltd., report no. ABT 401/024588. GLP, unpublished. | Y | Valent Biosciences |  |  |
| IIIB, 2.7.4/01 | Young, S. | 2003 | ‘VECTOBAC’ WG two year storage stability. Huntingdon Life Sciences Ltd., report no. ABT 401/024588. GLP, unpublished. | Y | Valent Biosciences |  |  |
| IIIB, 2.7.5/01 | Young, S. | 2003 | ‘VECTOBAC’ WG two year storage stability. Huntingdon Life Sciences Ltd., report no. ABT 401/024588. GLP, unpublished. | Y | Valent Biosciences |  |  |
| / | Anonymous | 2002 | VALENT BIOSCIENCES CORPORATION  COMMODITY SPECIFICATION DOCUMENT CN.76.0162  (Packaging\_drum.pdf) | / | Valent Biosciences |  |  |
| / | / | 2017 | VectoBac\_WG\_-\_Response\_document\_17\_11\_2017.docx | / | Valent Biosciences |  |  |
| / | / | 2020 | Microbiology screnning of Vectobac Water Dispersible Granule, Study number: QAQC-036, Performing laboratory: Valen BioSciences Quality Control Laboratory, Osage, Iowa USA | / | Valent Biosciences |  |  |
| / | Comb T. | 2018 | Vectobac WDG: spontaneity of dispersion | Y | Valent Biosciences |  |  |
| **Section 3** | | | | | |  |  |
| No study reports submitted | | | | | |  |  |
| **Section 4** | | | | | |  |  |
| No study reports submitted | | | | | |  |  |
| **Section 5** | | | | | |  |  |
| IIIB, 5.1/01 | Young, S. | 2003 | ‘VECTOBAC’ WG two year storage stability. Huntingdon Life Sciences Ltd., report no. ABT 401/024588. GLP, unpublished. | Y | Valent Biosciences |  |  |
| / | Winston Lin | 2015 | Bioburden analysis of Vectobac WDG, Performing laboratory project: 2539 SN1, Performing laboratory: IIT Research Institute Life Sciences Group, Chicago, Illinois, Sponsor: Valent BioSciences Corporation, GLP |  |  |  |  |
| **Section 6** | | | | | |  |  |
| IIIB, 6.1/01 | DeChant, P. | 2005 | A trial to Evaluate ‘VECTOBAC’ WDG in low volume ground application for control of container breeding species. Valent BioSciences. Report number 2003PDECH008. Dated 31.1.2005. Non-GLP. Unpublished. | Y | Valent Biosciences |  |  |
| IIIB, 6.1/02 | DeChant, P. | 2006 | A trial to evaluate the efficacy of aerially applied ‘VECTOBAC’ WDG for the control of *Ochlerotatus caspius* and *Culex spp.* larvae in rice fields under mid season rice growing conditions. Valent BioSciences. Report number 2003PDECH014. Dated 7.2.2006. Non-GLP. Unpublished. | Y | Valent Biosciences |  |  |
| IIIB, 6.1/03 | Muller, M.J. | 1999 | Testing of ‘VECTOBAC’ WDG Mosquito Control. Valent BioSciences. Report number 7627. Dated 8.2.1999. Non-GLP. Unpublished | Y | Valent Biosciences |  |  |
| IIIB, 6.1/04 | Rath, A. | 2000a | Report on aerial trial of ‘VECTOBAC’ WG. Brisbane City Council, Mosquito and Pest Services, Queensland, Australia. Report number 7667. Dated 02.2000. Non-GLP. Unpublished | Y | Valent Biosciences |  |  |
| IIIB, 6.1/05 | DeChant, P. | 1999 | ‘VECTOBAC’ WDG comparison to ‘VECTOBAC’ 12AS for control of *Aedes vexans.*. Abbot laboratories, Portland OR. Report number 16574. Dated 12.1999. Non-GLP. Unpublished | Y | Valent Biosciences |  |  |
| IIIB, 6.1/06 | Ballaux, J.C. | 2001 | A trial to compare the new ‘VECTOBAC’ WDG formulation with the reference ‘VECTOBAC’ 12AS for the control of *Aedes caspius* in the estuary of the Odiel river (Huelva). Estuario del Rio Odiel, Huelva, Spain. Report number 2000JBALL006. Dated 07.2001 Non-GLP. Unpublished | Y | Valent Biosciences |  |  |
| IIIB, 6.1/07 | DeChant, P. | 2001 | A trial to conduct large scale field trials with IcyBac delivery system to test effectiveness of this application methodology. Valent Biosciences. Report number 2000PDECH578. Dated 01.25.2002. Non-GLP. Unpublished | Y | Valent Biosciences |  |  |
| IIIB, 6.1/08 | Su, T. and Mulla, M.S. | 1999 | Field evaluation of new water dispersible granular formulations of *Bacillus thuringiensis* Ssp. *Israelensi and Bacillus sphaericus* against *Culex* mosquitoes in microcosms. Department of Entomology. University of California, Riverside. Journal of the American Mosquito Control Association. 15(3):356-365, 1999. Non-GLP. Published. | N | - |  |  |
| IIIB, 6.1/09 | Bouras, D. | 2007 | Efficacy comparison of different application rates of VECTOBAC WDG against *Ochlerotatus caspius* in rice fields. ANADIAG HELLAS Ltd. Greece, Trial number VAL-07-GR-29 Non-GLP. Unpublished | Y | Valent Biosciences |  |  |
| IIIB, 6.1/10 | Becker, N. | 2003 | Ice granules containing endotoxins of microbial agents for the control of mosquito larvae – a new technique. Journal of the American Mosquito control Association, 19(1): 63-66, 2003, Non-GLP. Published. | N | - |  |  |
| IIIB, 6.2/01 | DeChant, P. | 2002 | A trial Determine susceptibility of *Oc. japonicus* ‘VECTOBAC’ WDG and VectoLex WDG. Valent Biosciences. Unpublished report number 2001PDECH017. Dated 06.04.2002. Non-GLP. Unpublished | Y | Valent Biosciences |  |  |
| IIIB, 6.2/02 | Russell, T.L., Brown, M.D., Purdie, D.M., Ryan, P.A., Kay, B.H. | 2003 | Efficacy of ‘VECTOBAC’ (Bacillus thuringiensis variety israelensis) Formulations for Mosquito Control in Australia. J. Econ. Entomol. 96(6): 1786-1791 Non-GLP. Published. | N | - |  |  |
| IIIB, 6.2/03 | Rath, A. | 2000b | Laboratory study on ‘VECTOBAC’ water dispersible granules (WDG) (ABG6511) in comparison with liquid formulation ‘VECTOBAC’ 12AS using earthen jar method against vector mosquitoes in the tropical environment. Vector Control Research Unit, University Sains Malaysia, Penang, Malaysia. Report number 12668. Dated 06.2000. Non-GLP. Unpublished | Y | Valent Biosciences |  |  |
| IIIB 6.10.2-01 | Becker N. | 2003 | Ice granules containing endotoxins of microbial agents for the control of mosquito larvae – a New application technique. Becker report VECTOBAC WG\_Germany 2003 Report Not GEP  Published | N | VBC |  |  |
| IIIB 6.10.2-02 | Giordana C. | 2006 | Demonstrate efficacy of .VECTOBAC WG aerial application in rice fields. 2005PDECH014 Not GEP Unpublished | Y | VBC |  |  |
| IIIB 6.10.2-03 | Bouras D., Pantazis S. | 2006 | Efficacy comparison of different application rates of VECTOBAC WDG against *Culex pipiens* in simulated rice fields. 2006PDECH003 GEP Unpublished | Y | VBC |  |  |
| IIIB 6.10.2-04 | Bouras D. | 2008 | Efficacy comparison of different application rates of VECTOBAC WDG against *Ochlerotatus caspius* in rice fields. 2007PDECH007 GEP Unpublished | Y | VBC |  |  |
| IIIB 6.10.2-05 | Bouras D. | 2008 | Efficacy evaluation of different application rates of VECTOBAC WDG against *Anopheles* sp. on a laboratory study. VAL-09-GR-07 GEP Unpublished | Y | VBC |  |  |
| IIIB 6.10.2-06 | Alvarez J. S. | 2007 | Evaluation of the EFFICACY and SELECTIVITY of the new formulation VECTOBAC WDG for the control of MOSQUITO LARVAE in Spain in comparison to the registered formulation VECTOBAC 12 AS. 2007 HKOTT007 GEP Unpublished | Y | VBC |  |  |
| IIIB 6.10.2-07 | Rydzanicz K., Lone E., Kiewra D., DeChant P. | 2008 | Comparison of the efficacy of two different microbial control agents in an irrigation ditch system in Wroclaw (Poland). 2007PDECH031 Not GEP Unpublished | Y | VBC |  |  |
| IIIB 6.10.2-08 | Eritja R. | 2009 | Report on a field test on the efficiency of ground-based Larvicidal application by misting a formulation of *Bacillus thuringiensis isralensis* (VECTOBAC WDG) on A*edes albopictus.* 2008HKOTT005a GEP Unpublished | Y | VBC |  |  |
| IIIB 6.10.2-09 | Eritja R. | 2010 | Report on a field test on the efficiency of ground-based Larvicidal application by misting of VECTOBACT WG, a formulation based on *Bacillus thuringiensis israelensis* (Strain AM65-52) ON *Aedes albopictus*. 2008HKOTT005 Study2 GEP Unpublished | Y | VBC |  |  |
| IIIB 6.10.2-10 | Besnard G., Kotter H., Foussadier R. | 2008 | Evaluation of a water dispersible granule formulation of Bti for Low Volume or Ultra Low Volume area spraying against mosquito larvae in woodland conditions. 2008HKOTT011-Study 1 Not GEP Unpublished | Y | VBC |  |  |
| IIIB 6.10.2-11 | Besnard G., Kotter H., Foussadier R. | 2008 | Evaluation of a water dispersible granule formulation of Bti for Ultra Low Volume area spraying against container breeding mosquito *Culex pipiens*. 2008HKOTT011-Study 2 Not GEP Unpublished | Y | VBC |  |  |
| IIIB 6.10.2-12 | Besnard G., Kotter H., Foussadier R. | 2008 | Evaluation of a water dispersible granule formulation of Bti for Ultra Low Volume area spraying against container breeding mosquito *Culex pipiens*. Second barn trial. 2008HKOTT011-Study 3 Not GEP Unpublished | Y | VBC |  |  |
| IIIB 6.10.2-13 | Mosca A. | 2008 | Evaluation of VECTOBAC WG misting treatments.  2008HKOTT018a  GEP  Unpublished | Y | VBC |  |  |
| IIIB 6.10.2-14 | Niclot M.V., Vitale M. | 2009 | Evaluation of wide area application of VECTOBAC WDG for the control of Aedes albopictus and other container breeding mosquitoes in Italy. 2008HKOTT018-Study 2 Not GEP Unpublished | Y | VBC |  |  |
| IIIB 6.10.2-15 | Becker N. | 2011 | Evaluation of VECTOBAC WG for control of *Aedes* spp. in woodland pools in Germany.  2010PDECH010a  GEP  Unpublished. | Y | VBC |  |  |
| IIIB 6.10.2-16 | Ruiz Contrera S., Caceres Benavides F. | 2001 | Ensayo de campo de formulaci6n Vectabac WDG (3000 JTII) frernte a *Aedes caspius* y comparaci6n con la forrnulaeiiin VECTOBAC 12 AS (1200 ITU). 2000JBALL006 Not GEP Unpublished | Y | VBC |  |  |
| IIIB 6.10.2-17 | Bellec ,S., Bousquet, C., Lagneau,,Ch. | 2012 | Evaluation de l’efficacité du VECTOBAC® WG versus VECTOBAC® 12AS par épandage aérien en conditions opérationelles sur larves *d’Aedes caspius* (Diptera: Culicidae) GEP No Unpublished | Yes | VBC |  |  |
| IIIB 6.10.2-18 | Faulde M.K., Scharninghausen J.J., Tisch M. | 2008 | Fire fighting truck-based emergency mosquito biolarviciding to prevent outbreaks of malaria and arboviral disease in Kabul, Afghanistan J. Pest Sci (2008) 81:71-77 Not GEP Published | No | -- |  |  |
| IIIB 6.10.2-19 | Majambere S. | 2007 | Microbial larvicides for malaria control in The Gambia Malaria Journal 2007, 6:76 Not GEP Published | No | -- |  |  |
| IIIB 6.10.2-20 | Dambach P. *et al* | 2014 | Efficacy of *Bacillus thurigiensis* var. *Israelensis* against malaria mosquitoes in northwestern Burkina Faso Parasites & Vectors 2014, **7**:371 Not GEP Published | No | -- |  |  |
| IIIB 6.10.2-21 | Fillinger U., Knols B.G.J., Becker N | 2003 | Efficacy and efficiency of new Bacillus thuringiensis var. Israelensis and Bacillus sphaericus formulations against Afrotropical anophelines in Western Kenya Tropical Medicine and Int’l Health, Vol 8, N° 1, 37-47 Not GEP Published | No | -- |  |  |
| IIIB 6.10.2-22 | Anon (ACFA) | 2012 | Evaluation of VECTOBAC G and VBC 60233 at rates of 3, 6 and 9 kg/ha for *Anopheles* spp. control  Report 2012HKOTT010\_Report\_1 Not GEP Unpublished | Yes | VBC |  |  |
| **Section 7** | | | | | |  |  |
| IIIB, 7.1.1/01 | Shults, S.K. and Watson, M. | 1997a | Acute oral toxicity (LD50) study in rats with ‘VECTOBAC’ WDG; Ricerca, Inc., Painesville, Ohio, USA. Report No. 7253-97-0111-TX-001, GLP. Unpublished | Y | Valent Biosciences |  |  |
| IIIB, 7.1.2/01 | Bennick, J. | 1997 | ‘VECTOBAC’ WDG (ABG-6490) Lot 30-058-BR, Acute Inhalation Toxicity Study in Rats, Stillmeadow, Inc., Sugar Land, Texas, USA.  Report No. 3567-97, GLP. Unpublished | Y | Valent Biosciences |  |  |
| IIIB, 7.1.3/01 | Shults, S.K. and Watson, M. | 1997b | Acute dermal toxicity (LD50) study in Albino Rabbits with ‘VECTOBAC’ WDG (ABG-6490); Ricerca, Inc., Painesville, Ohio, USA. Report No. 7253-97-0112-TX-001, GLP. Unpublished | Y | Valent Biosciences |  |  |
| IIIB, 7.2.1/01 | Shults, S.K. and Watson, M. | 1997c | Primary Dermal Irritation Study in Albino Rabbits with ‘VECTOBAC’ WDG (ABG-6490); Ricerca, Inc., Painesville, Ohio, USA. Report No. 7253-97-0114-TX-001, GLP. Unpublished | Y | Valent Biosciences |  |  |
| IIIB, 7.2.2/01 | Shults, S.K. and Watson, M. | 1997d | Primary Eye Irritation Study in Albino Rabbits with ‘VECTOBAC’ WDG (ABG-6490); Ricerca, Inc., Painesville, Ohio, USA. Report No. 7253-97-0113-TX-001, GLP. Unpublished | Y | Valent Biosciences |  |  |
| IIIB, 7.2.3/01 | Kuhn, J.O. | 2001 | ABG-6511, ‘VECTOBAC’ WDG, Lot 60-068-BR. Guinea pig maximization test for topically applied test substance. Stillmeadow, Inc., Sugarland, Texas, USA Report No. 6281-01, GLP. Unpublished | Y | Valent Biosciences |  |  |
| **Section 8** | | | | | |  |  |
| No study reports submitted | | | | | |  |  |
| **Section 9** | | | | | |  |  |
| No study reports submitted | | | | | |  |  |
| **Section 10** | | | | | |  |  |
| IIIB, 10.3/01 | Bocksch, S | 2006 | Assessment of side effects of VECTOBAC WG to the honey bee *Apis mellifera* L. in the laboratory limit test.  GAB Biotechnologie GmbH, Report No: 20061012/S1-BLEU GLP. Unpublished | Y | Valent Biosciences |  |  |
| Biodiversity | Aletru F | 2012 | Évaluation des effets éventuels de la préparation larvicide issue du *Bacillus thuringiensis israelensis* sur l’abeille domestique *Apis mellifera* m.  EID Atlantique / Rochefort sur Mer. Non-GLP, published | N | N/A |  |  |
| Biodiversity | Becker N. | 1998 | The use of Bacillus thuringiesis subsp israelensis (BTI) against mosquitoes, with special emphasis on the ecological impact.  Israel Journal of Entomology, Vol. xxxn (1998) pp. 63-69  Non-GLP, published | N | N/A |  |  |
| Biodiversity | Caquet T. *et al* | 2011 | Effects of repeated field applications of two formulations of Bacillus thuringiensis var. israelensis on non-target saltmarsh invertebrates in Atlantic coastal wetlands  Elsiver press, Ecotox and Env. Safety, 74 (2011) 1122-1130. Non-GLP, published | N | N/A |  |  |
| Biodiversity | Duchet C. *Et al* | 2010a | Population-level effects of spinosad and Bacillus thuringiensis israelensis in Daphnia pulex and Daphnia magna: comparison of laboratory and field microcosm exposure conditions  Ecotoxicology (2010) 19:1224–1237  Non-GLP, published | N | N/A |  |  |
| Biodiversity | Duchet C. *Et al* | 2010b | Influence of environmental factors on the response of a natural population  of Daphnia magna (Crustacea: Cladocera) to spinosad and  Bacillus thuringiensis israelensis in Mediterranean coastal wetlands  Environmental Pollution 158 (2010) 1825–1833  Non-GLP, published | N | N/A |  |  |
| Biodiversity | Duchet C. *Et al* | 2011 | Chitobiase activity as an indicator of altered survival, growth and reproduction in Daphnia pulex and Daphnia magna (Crustacea: Cladocera) exposed to spinosad and diflubenzuron  Ecotoxicology and Environmental Safety 74 (2011) 800–810  Non-GLP, published | N | N/A |  |  |
| Biodiversity | Duchet C. *Et al* | 2008 | Effects of spinosad and Bacillus thuringiensis israelensis on a natural population of Daphnia pulex in field microcosms  Chemosphere 74 (2008) 70–77  Non-GLP, published | N | N/A |  |  |
| Biodiversity | EID Atlantique | 2010 | Rapport annuel 2010 de l’EID Morbihan  Non-GLP, published | N | N/A |  |  |
| Biodiversity | Franquet E. and  Fayolle S. | Un-known | Etude d’impact d’un éventuel traitement au B.t.i. sur le territoire du Parc naturel régional de Camargue. Rapport scientifique  Facultés des Sciences et Techniques de St Jérôme, UDESAM, Marseilles  Non-GLP, published | N | N/A |  |  |
| Biodiversity | Guidi V. *Et al* | Un-known | Distribution of Bacillus thuringiensis subsp. israelensis in Soil of a Swiss Wetland Reserve after 22 Years of Mosquito Control  Applied and Environmental Micorbiology, June 2011, p. 3663–3668  Non-GLP, published | N | N/A |  |  |
| Biodiversity | Guidi V. *Et al* | Un-known | A real-time PCR method to quantify spores carrying the Bacillus thuringiensis var. israelensis cry4Aa and cry4Ba genes in soil  The Society for Applied Microbiology, Journal of Applied Microbiology 109 (2010) 1209–1217  Non-GLP, published | N | N/A |  |  |
| Biodiversity | Lagadic L | 2013 | Bti sprays do not adversely affect non-target aquatic invertebrates in French Atlantic coastal wetlands  Journal of Applied Ecology 2013  Non-GLP, published | N | N/A |  |  |
| Biodiversity | Lagadic L | 2011 | Évaluation à long terme du risque écotoxicologique des traitements de démoustication  Réunion du Conseil Scientifique et technique de l’EID Atlantique  Rochefort – 19 Mai 2011  Non-GLP, published | N | N/A |  |  |
| Biodiversity | Lagadic L | 2009 | Avis concernant les effets comparés du VECTOBAC® 12AS et du VECTOBAC® WG sur les communautés d’invertebrés aquatiques des zones humides littorales du Morbihan  Équipe Écotoxicologie et Qualité des Milieux Aquatiques  UMR INRA-Agrocampus Ouest 985, Écologie et Santé des Ecosystèmes  Non-GLP, published | N | N/A |  |  |
| Biodiversity | Le Goff P. et al | 2009 | Évaluation à long terme des effets de la démoustication dans le Morbihan suivi de l’impact écotoxicologique d’une nouvelle formulation de larvicide sur les invertébrés aquatiques : étude comparative entre VECTOBAC® WG et VECTOBAC® 12AS  Équipe Écotoxicologie et Qualité des Milieux Aquatiques  UMR INRA-Agrocampus Ouest 985, Écologie et Santé des Ecosystèmes  Non-GLP, published | N | N/A |  |  |
| Biodiversity | Lundstrom J. | 2010 | Production of wetland Chironomidae (Diptera) and the effects of using Bacillus thuringiensis israelensis for mosquito control  Bulletin of Entomological Research (2010), 100:117-125  Non-GLP, published | N | N/A |  |  |
| Biodiversity | Lundstrom J. | 2009 | Are there any long-term indirect ecological effects of using Bti against flood-water mosquito larvae in temporary wetland environments?  Upsala University  Non-GLP, published | N | N/A |  |  |
| Biodiversity | Moulinier Cl. *Et al* | 1981? | Etude de l’innocuité du Bacillus thuringiensis var. israelensis pour les larves d’huitres  Bulletin de la société de pathologie exotique  Non-GLP, published | N | N/A |  |  |
| Biodiversity | Timmermann U. and Becker N. | 2003 | Die Auswirkung der Stechmückenbekämpfung auf die Ernährung auenbewohnender Vogelarten  Carolinea 61 (2003) 145-165, 6 abb., Karlsruhe.  Non-GLP. Published | N | N/A |  |  |
| **Section 11** | | | | | |  |  |
| No study reports submitted | | | | | |  |  |
| **Section 12** | | | | | |  |  |
| No study reports submitted | | | | | |  |  |

Annex 3: Analytical methods residues – active substance

*Bacillus thuringiensis israelensis* serotype H-14 strain AM65-52

Date: 29.12.2014

**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 4 : Toxicology and metabolism –active substance

< *Bacillus thuringiensis israelensis* AM65-52 >

Threshold Limits and other Values for Human Health Risk Assessment

Date: 29.12.2014

| **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 Dir. 67/548/EEC) | Not relevant |
| with regard to toxicological data (according to the criteria in Reg. 1272/2008) | Not relevant |

Annex 5 : Toxicology – biocidal product

< VECTOBAC WG>

Date: 29.12.2014

|  |  |
| --- | --- |
| General information | |
| Formulation Type | WG |
| Active substance(s) (incl. content) | *Bti* AM 65-52 (37.4% 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) | > 5000 mg/kg bw |  |  |  |
| Rat LD50 dermal (OECD 402) | > 5000 mg/kg bw |  |  |  |
| Rat LC50 inhalation (OECD 403) | > 0.014 mg/L |  |  |  |
| Skin irritation (EPA 81-5) | Not skin irritant |  |  |  |
| Eye irritation (OECD 405) | Not eye irritant |  |  |  |
| Skin sensitisation (OECD 406) | Not skin sensitizer |  |  |  |

| Additional toxicological information (e.g. Annex IIIB, point 6.5, 6.7) | | | | |
| --- | --- | --- | --- | --- |
| 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 (Annex IIIB, point 9) | |
| Directive 1999/45/EC | None |
| Regulation 1272/2008/EC | None |

Annex 6 : Safety for professional operators

< VECTOBAC WG>

Date: 29.12.2014

Exposure assessment

| Exposure scenarios for intended uses (Annex IIIB, point 6.6 ) |
| --- |

No models are currently available to estimate professional exposure from the application of micro-organisms. In this context, the exposure has been qualitatively estimated.

The product is intended to be applied by ground (portable pump pressure sprayer, motorized portable blower, vehicle mounted mototrized spray equipment) or aerial equipment (spraying or granules spreader).

The typical routes of exposure are from dermal absorption, inhalation and ingestion. The potential for 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, VECTOBAC WG 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.

Annex 7 : Safety for non-professional operators and the general public

<VECTOBAC WG>

Date:29.12.2014

| General information | |
| --- | --- |
| Formulation Type | WG |
| Active substance(s) (incl. content) | *Bti* (37.4% w/w technical slurry) |
| Category | PT18 |

| **< *Bti* AM 65-52>** |
| --- |

| Data base for exposure estimation | |
| --- | --- |
| according to | Appendix: Toxicology and metabolism – active substance/CAR |

| Exposure scenarios for intended uses (Annex IIIB, point 6.6 ) | |
| --- | --- |
| Primary exposure | Not relevant |
| Secondary exposure, acute | Bystander and resident exposure (inhalation and oral); worker exposure (dermal) |
| Secondary exposure, chronic | Not relevant |

Conclusion:

Exposure of non-professionals is not relevant.

Indirect exposure

* Ground application:

Ground spray application could lead to an exposure to the spray drift, if a bystander is walking next to a area being treated. Bystanders are excluded from treated areas to ensure only protected professionals can possibly be exposed to VECTOBAC WG. 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 VECTOBAC WG as biocidal product applied with an aerial equipment This is also considered applicable when VECTOBAC WG is applied with vehicle mounted motorized spray equipment taking into account the expected high spray drift. Therefore, another mode of application should be envisaged for the treatment of areas closed to habitations.

Finally, in case of re-entry after treatment on rice, it is recommended to workers to wear a coverall and gloves. In this context, the risk is considered low.

Annex 8 : Residue behaviour

*Bacillus thuringiensis israelensis* strain AM65-52

Date: 22.01.2015

Intended Use (critical application)

Active substance(s): *Bacillus thuringiensis israelensis* strain AM65-52 (37.8% w/w)

Formulation of biocidal product: Aqueous suspension concentrate

Place of treatment: outdoor, applied using conventional ground or aerial application equipment on water where mosquitoes breed.

Control of mosquito larvae in water where mosquito breeding occurs.

Bti AM65-52 is a larvicide and the timing of application will depend on the level of larvae infestation and growth stage. The product should be applied during the first to the 4th larval instar with 0.125-1 kg/ha with intervals of 7 days, up to 8 applications per season

The product is to be used for control of mosquito and black fly larvae in water habitats (i.e. Irrigation ditches, reservoirs, lakes, rivers, flood plains, rice fields, canals, marshland, ponds, catch basins, drainage and roadside ditches, waters in irrigated crops, waste water, sewage effluent/lagoons, septic ditches, animal waste lagoons, natural/manmade containers). The intended use descriptions of the Bti AM65-52-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 water in irrigated crops).

The intended use descriptions of the Bti AM65-52-containing biocidal products for which authorisation is sought indicate that these uses could lead to an exposure via food consumption. As aerial applications are also intended in water surrounding rice crops, surfaces where food crops are implanted could be contaminated (ie private vegetable garden).

*Bacillus thuringiensis* has recently 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.

In 2013 ANSES published an opinion on risk related to application of *Bacillus thuringiensis* as insecticide (ANSES, Saisine n°2013-SA-0039) and concluded that for re-registration of products based on *Bacillus thuringiensis* the following data have to be submitted for each strain:

* specific identification method,
* data on the toxicological potential,
* specific residue data

A request has been made to the applicant to fulfill this data gap.

The following argumentation has been submitted by the applicant:

*”Use of VECTOBAC WG is requested in water surrounding rice (into paddy water). It should be remembered the rice paddies contain stagnant water that is an important area for proliferation of mosquito larvae and contains numerous other contaminants and a large natural microbial load. However, other points of consideration is that VECTOBAC WG (as liquid application) will only be sprayed on the water when the rice plants are small, as if the vegetation is high this formulation would not be appropriate for application as the spray would partially be intercepted by the vegetation thus not reaching the targeted mosquito larvae in the water. Once the vegetation becomes more dense, applications are usually made with granular formulations that can penetrate the vegetation. Notwithstanding the information above it should also be remembered that mosquitoes proliferate in water and towards the end of the rice growing period the fields are dried approximately 4 weeks before the grain harves, thus permitting plenty of time for the Bti to degrade due to UV lightt. Rice grains are also covered by a husk that is removed prior to consumption.”*

*Bti* AM65-52 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 VECTOBAC WG 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.

VECTOBAC WG 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 surrounding rice crops can reach food crops located nearby, risk of oral exposure to residues after application via residues of Btk on food crops is considered to be negligible. Therefore, the use of VECTOBAC WG in water surrounding rice, with a pre harvest interval of 1 month is acceptable.

However, no data nor justification have been given for waters in irrigated crops excepting rice. As indirect exposure via food cannot be excluded in those cases and without further information, the application of VECTOBAC WG in waters in irrigated crops is not supported.

Annex 9: Efficacy of the active substance from its use in the biocidal product (\*)

| Test substance | Test organisms | | Test system / Concentrations applied / exposure time | Test conditions | Test results: effects, mode of action, resistance | Reference | Reliability |
| --- | --- | --- | --- | --- | --- | --- | --- |
| VECTOBAC WDG | *Aedes vexans* (L2/L3, *Culiseta annulata* (L1/L4 ), *Ochlerotatus sticticus* ( L2/L3) et *Aedes rossicus* (L2/L3) | | Three mixtures were prepared using 10 litres of tap water mixed with 100, 200 and 400 g of VECTOBAC WDG, respectively, each for 1 ha. A fourth mixture was prepared using 5 litres of tap water mixed with 400 g of VECTOBAC WDG to treat 1 ha. These mixtures were then used to produce the IcyPearls. The granules were applied via an insulated bucket equipped with a rotating device (seeder) and operated by the pilot in a Bell 47 helicopter.  First treatment site: 10 kg IcyPearls/ha containing 100 g VECTOBAC WDG  Second treatment site: 10 kg IcyPearls/ha containing 200 g VECTOBAC WDG  Third treatment site: 10 kg IcyPearls/ha containing 400 g VECTOBAC WDG  Fourth treatment site: 5 kg IcyPearls/ha containing 400 g VECTOBAC WDG | Natural conditions | The VECTOBAC WDG showed a mortality greater than 90 % at the rate of 200 g/ha and 400 g/ha of product in 10 kg (120 IUT/mg) and in 5 kg (240 IUT/mg) of IcyPearls/ha after 48 hours of application. | IIIB.5.10/01 | 2 |
| VECTOBAC WG | *Aedes caspius* | | Field trial conducted in rice fields in Novara, Piedmont, Italy. The fields were post-flood L1-4 stages, rice variety was Arborio.  Schweizer 300C airship equipped with boom and nozzle system. Applied aerially at 40 L/ha in on 16 June 2005.  Application rates were 0.35 and 0.4 kg/ha equivalent to 1.1 x 109 and 1.2 x 109 ITU as/ha | No details reported. | The following remarks are extracted from the doc IIIB6.1-02. This study was submitted for the inclusion of the Bti AM 65-52 in annex I. It was not accepted by the RMS for the reasons cited below:  The original study is unreadable: no explanation about materiel and methods adopted; aeriel sprays generally require an “ad hoc” formulation; data are shown in a row way and are in Italian; no statistical treatment of data was shown; in the goal of the work there is the control of *Ochlerotatus caspius* and *Culex spp*, but the results are referred only to the former species. It’s not clear if the experiment was performed with WG or WDG formulation. | IIIB.5.10/02 | 4 |
| VECTOBAC WDG | *Culex pipens* (L2/L3) | A simulated field trial was conducted using four rates of VECTOBAC WDG applied by spray on a mixed age population of *Culex pipiens* (L2/L3) larvae in a simulated rice field in at rates of 125, 250, 500 and 1000 g/ha. The application was performed with a boom sprayer atomiser, with a water volume of 400 l/ha. | | Water temperature ranged between 30.6 and 32.7°C, air temperature between 30 and 32°C 24 and 48 hours after application. | The VECTOBAC WDG showed a mortality greater than 90 % at the rate of 125 g/ha, 250 g/ha, 500 and 1000 g/ha after 24 hours of application. | IIIB.5.10/03 | 2 |
| VECTOBAC WDG | *Anopheles sp* (L2/L3) | | The second and third stage larvae of *Anopheles sp* were in contact with the VECTOBAC WG at rate of 0.125, 0.25, 0.5 and 1 ppm. The number of dead and live larvae was assessed in each container at 24 and 48 hours after of application.  . | Natural conditions | The VECTOBAC WDG showed a mortality greater than 90 % at the rate of 0.125, 0.25, 0.5 and 1 ppm after 48 hours of application | IIIB.5.10/05 | 2 |
| VECTOBAC WDG  VECTOBAC 12AS | *Aedes caspius* | | A field trial was conducted using two rates of VECTOBAC WDG and one of VECTOBAC 12AS (applied as a reference). The test materials were applied to a tidal swamp area in Spain at rates of 500 and 1000 g/ha for VECTOBAC WDG and 2500 g/ha for VECTOBAC 12AS. The application was made with a manual backpack sprayer, using a volume of 100 L/ha Effects on a natural population of *A. caspius* larvae were observed 24 and 48 hours after application. An untreated control was used for comparison. | Water temperature range between 22 and 38.5 °C | The VECTOBAC WDG showed a mortality greater than 90 % at the rate of 500 g/ha, 1000 g/ha 2500 g/ha after 24 hours of application | IIIB.5.10/06 | 2 |
| VECTOBAC WDG | *Culex pipiens* | | A field trial was conducted using 3 rates of VECTOBAC WDG. The test material was applied to an irrigation ditch area in Poland using a hand operated compression sprayer at rates of 200, 400 and 800 g/ha. Effects on the natural population of *Culex pipiens* larvae were observed for 14 days after application. An untreated control was used for comparison. | Natural conditions | The VECTOBAC WDG showed a mortality greater than 90 % at the rate of 200 g/ha after 7 days, 400 g/ha after 4 days and 800 g/ha after 2 days of treatment. | IIIB.5.10/07 | 2 |
| VECTOBAC WDG | *Aedes albopictus* | | A semi-field trial was conducted using VECTOBAC WDG at a rate of 1000 g/ha diluted in 200, 300 and 500 L of water. The test material was applied using a low volume mist sprayer (Martignani B-748) mounted on a 4x4 pickup Ford Ranger. The test site was a disused camp site using five empty bungalows. The target water bowls are set to receive a theoretical dose of 7 mg VECTOBAC WDG per bowl under field conditions. The larvicidal effect of the actual amount reaching the bowls in the field was then evaluated by the addition of stock reared larvae in the laboratory. Six locations were selected to place the bowls in the houses. They were placed perpendiculary to the application path at several distances. Effects on laboratory bred *Aedes albopictus* larvae were observed 24 and 48 hours after application. | Temperature was 26 °C and relative humidity ranged between 45 and 58% | The VECTOBAC WDG showed a mortality rate greater than 90 % until 21 m | IIIB.5.10/08 | 2 |
| VECTOBAC WDG | *Aedes albopictus* | | A semi-field trial was conducted using VECTOBAC WDG at a rate of 1000 g/ha at diffent flow rates (50, 100 and 200 L/h) of water. The test material was applied using a low volume mist sprayer (Martignani B-748) mounted on a 4x4 pickup Ford Ranger. The test site was a disused camp site using five empty bungalows. Bowls were placed at various distances (filled and empty) along this distance, 9.0, 13.7, 15.9, 18.5, 19.5 and 28.1 meters. | Natural conditions | The VECTOBAC WDG showed a mortality greater than 90 % until 28.1. | IIIB.5.10/09 | 2 |
| VECTOBAC WG | *Culex pipiens* (L3) | | Semi-field trial conducted in two mixed woodland areas. The application was made using a Martignani sprayer mounted on a 4x4 pickup with either a low volume or ultra low volume nozzle. Application was made to either dense undergrowth with lots of shrubs or thin undergrowth without shrubs at a rate of 1000 g/ha. In total four treatments were made, 100 and 200 l/h at both low and ultra low volume. | Temperature ranged between 23 and 29 °C and relative humidity ranged between 64 and 80% | We couldn’t conclude because there was no table of results showing the mortality (%) with the two flow rates of application of the product and the graph presented was not clear : no indication was mentioned in order to distinguish between the two flow rates tested | IIIB.5.10/10 | 3 |
| VECTOBAC WG | *Culex pipiens* (L3) | | A field trial was conducted using VECTOBAC WDG at a rate of 1000 g/ha applied with different flow rates (200, 300 and 400 L/h) at several distances. The test material was applied using an ultra-low volume mist sprayer on a 4x4 pickup, vehicle at 200 l/h. The test site was an isolated house. Effects on *C. pipiens* larvae were observed 24 hours after application. | Not reported. | The VECTOBAC WDG showed a mortality greater than 90 % until 15 m. | IIIB.5.10/11 | 2 |
| VECTOBAC WG | *Culex pipiens* (L3) | | A field trial was conducted using VECTOBAC WDG at a rate of 1000 g/ha applied with two flow rates (100 and 200 L/h) at several distances. The test material was applied using an ultra-low volume mist sprayer on a 4x4 pickup, vehicle at 100 and 200 l/h. The test site was an isolated barn. Effects on *C. pipiens* larvae were observed 24 hours after application. | Not reported. | The VECTOBAC WDG showed a mortality greater than 90 % until 30 m. | IIIB.5.10/12 | 2 |
| VECTOBAC WG | *Culex pipiens* (L3) | | A semi - field trial was conducted using VECTOBAC WDG at rates of 250 and 500 g/ha applied with two flow rates (16.9 and 7.9 L/min). The test material was applied using an ultra-low volume mist sprayer Tifone City 460-35, Hp engine and a pump. Bowls containing larvae of *C. Pipiens* were placed at two levels of ground (50 cm and 4 m). The test site was an abandoned farm house. Effects on field collected *C. pipiens* larvae were observed 24 and 48 hours after application. | Not reported. | The VECTOBAC WDG showed a mortality greater than 90 % at the rate of 500 g/ha (9.7 L/min) at the level of 50 cm after 60 hours of treatment. | IIIB.5.10/13 | 2 |
| VECTOBAC WDG | *Aedes albopictus* (L2/L3)  *Culex pipiens* (L2/L3) | | A field trial was conducted using VECTOBAC WDG. The test material was applied in a cemetery at the rate of 1 kg/ha with plots in three areas; burial niches on the perimeter wall, underground burial niches, and grave fields. The product was sprayed at several distances from the site of application and from several levels of the ground. An untreated control was used for comparison. The application was by motorised backpack blower (burial niches and grave fields) and truck mounted sprayer (grave fields). | The test was carried out from the late afternoon to the night to avoid fast evaporation due to high air temperature and low humidity during the day. | The VECTOBAC WDG showed a mortality greater than 90 % at different levels from the ground tested and until 15 m from the site of the spraying. | IIIB.5.10/14 | 2 |
| VECTOBAC WG | *Aedes* (*Ochlerotatus*) *cantans*; *Aedes* (*Ochlerotatus*) *communis*; *Aedes cinereus* and *Aedes* (*Ochlerotatus*) *punctor* | | Woodland pools infested with naturally occurring mosquito larvae (*Aedes* genus) were treated with the product VECTOBAC WDG at the rates of 125, 250 and 500 g/ha.Treatments were made to which the appropriate pre-determined amount of VECTOBAC® WG had been added. This was then suspended in 500 mL of filtered water and evenly distributed on the surface of the pool by a 1 litre hand pump.  At each sampling site the larvae was counted by stage (L1/2, L3 and L4) at pre-treatment and at 24, 48, 72, 96, 120, 144 and 168 hours after treatment.  . | Water temperature was approx. 12.3°C | The VECTOBAC WDG showed a mortality greater than 90 % after 144 hours at 125 g/ha and 96 hours at 250 g/ha and 500 g/ha after 24 hours of treatment. | IIIB.5.10/15 | 2 |
| VECTOBAC WDG | *Aedes caspius* (L3) | | A field trial was conducted using two rates of VECTOBAC WDG. The test material was aerially applied to salt marshes in Spain at rates of 0.50 and 1.0 kg/ha. Effects on a mixed age population of *A. caspius* larvae, but principally L3 were observed 24 and 48 hours after application. An untreated control plus a standard of VECTOBAC 12AS was used for comparison. | Water pH 7-8. Water temperature Min 17ºC – Max 36ºC; Water conductivity 41,6 – 65,6; Measure of vegetation and water depth | This study was submitted for the inclusion of the Bti AM 65-52 in annex I. It was not accepted by the RMS because the  data were missing | IIIB.5.10/16 | 4 |
| VECTOBAC WG | *Aedes* (*Ochlerotatus*) *caspius* | | A field trial was conducted using two rates of VECTOBAC WG and one trial of VECTOBAC 12AS. The test material was aerially applied using an airplane equipped with boom and nozzle system at rates of 750 and 1.0 kg/ha for VECTOBAC WG and 1.7L/ha for VECTOBAC 12AS. Effects on a mixed age population of *A. caspius* larvae were observed 24 and 48 hours after application. | Degree of infestation, vegetation, temperature of water and air, height of water were all measured. | The VECTOBAC WG showed a mortality greater than 90 % at the rate of 1000 g/ha after 24 hours of treatment. | IIIB.5.10/17 | 2 |

*(\*) fill in one table for each MG/PT and/or field of use envisage*

1. Please insert additional columns as necessary [↑](#footnote-ref-1)
2. Please insert additional columns as necessary [↑](#footnote-ref-2)
3. WHO/CDS/WHOPES/GCDPP/2005.13 Guidelines for laboratory and field testing of mosquito larvicides. [↑](#footnote-ref-3)
4. 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-4)
5. Rautmann D., Streloke M., Winkler R., 1999. New basic drift values in the authorization procedure for plant protection products. Workshop on risk assessment and risk mitigation measures (WORMM), 27-29 September 1999. [↑](#footnote-ref-5)
6. 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-6)
7. [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-7)