Kemikalieinspektionen
Swedish Chemicals Agency

Authorisations and Guidance

Product Assessment Report
Related to product authorisation under Regulation (EU) No 528/2012

VectoBac G and VectoBac GR

Type of application Re-authorisation	Product type Insecticide PT18
Swedish authorisation number 4889, 4924	Date of decision/Entry into force 01 May 2015
Active substance Bacillus thuringiensis subspecies israelensis serotype H-14, strain AM65-52, 2.8 % (w/w)	Date of expiry 30 April 2025
Sweden's R4BP reference code 2013/26755/7930/SE/ANP/11088 2013/26755/7930/SE/APP/11090	User category Trained professional

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1 GENERAL INFORMATION ABOUT THE PRODUCT APPLICATION

1.1 APPLICANT

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Postal Code:	69771
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1.1.1 Person authorised for communication on behalf of the applicant

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1.2 CURRENT AUTHORISATION HOLDER[1]

Company Name:	Sumitomo Chemical Agro Europe SAS (same as applicant)
Address:	Parc d'Affairs de Crécy, 2 rue Claude Chappe
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[1] Applies only to existing authorisations

E-mail address:	Sylvia.plak@sumitomo-chem.fr
Letter of appointment for the applicant to represent the authorisation holder provided (yes/no):	Not applicable

1.3 PROPOSED AUTHORISATION HOLDER

Company Name:	Sumitomo Chemical Agro Europe SAS (same as applicant)
Address:	Parc d'Affairs de Crécy, 2 rue Claude Chappe
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Letter of appointment for the applicant to represent the authorisation holder provided (yes/no):	Not applicable

1.4 INFORMATION ABOUT THE PRODUCT APPLICATION

Application received:	30th of August 2013
Application reported complete:	4th of March 2014
Type of application:	Reregistration
Further information:	Applicant has indicated submission of application for mutual recognition in RO, SK, HU, IT, FR, DE, CZ, BG, AT, CH, ES and PT.

1.5 INFORMATION ABOUT THE BIOCIDAL PRODUCT

1.5.1 General information

Trade names:	VectoBac G and VectoBac GR
Manufacturer's development code number(s), if appropriate:	ABG-6189=VBC-60241 (VectoBac G) and ABG-60233 (VectoBac GR)
Product type:	PT 18
Composition of the product (identity and content of active substance(s) and substances of concern; full composition see Confidential Important Information document):	VectoBac G and VectoBac GR contain at average 2.8% w/w *Bti* Strain AM65-52 as the active microorganism.
Formulation type:	VectoBac G and VectoBac GR are granule formulations.
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); 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

1.5.2 Information on the intended use(s)

1.5.2.1 Uses claimed by the applicant

Overall use pattern (manner and area of use):	Intended for the control of mosquito larvae in water habitats such as (but not limited to) irrigation ditches, reservoirs, lakes, rivers, canals, marchland, ponds, catch basins, drainage and roadside ditches, waters in irrigated crops, waste water, sewage effluent/lagoons, septic ditches, animal waste lagoons, natural/manmade containers. Ground application as a coarse surface spray, but can also be applied aerially.
Target organisms:	Mosquito larvae
Category of users:	Professional
Further information (if any)	VectoBac G and VectoBac GR is only effective at growth stages 1-4. The dosage (2.5-15 kg/ha) is dependent on larval density and water quality, and when larvae at stage 4 are dominant a higher dose is required. 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, and/or significant water exchange, the high rate should be used to provide good control of mosquitoes. VectoBac G and VectoBac GR should not be applied to food and water directly intended for human consumption.

1.5.2.2 Uses authorised by the Reference member state

Overall use pattern (manner and area of use):	Control of mosquito larvae in water habitats. Ground application as a coarse surface spray, but can also be applied aerially. 2.5-15 kg/ha depending on the target species population density and the water quality of the habitat. For ground applications there is an annual limit of 8 times with an interval of at least 7 days between.

	For aerial applications there is an annual limit of 4 times with an interval of at least 14 days between.
Target organisms/ stages:	Mosquito larvae Culicidae including species of the Genera: *Ochlerotatus sp.*, *Aedes sp.*, *Culex sp.*, *Culiceta sp*. and *Anopheles sp*. Development stage: Larvae
Category of users:	Trained professional
Further information (if any)	VectoBac G and VectoBac GR are only effective at growth stages 1-4. The dosage (2.5-15 kg/ha) is dependent on larval density and water quality, and when larvae at stage 4 are dominant a higher dose is required. 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, and/or significant water exchange, the high rate should be used to provide good control of mosquitoes. VectoBac G and VectoBac GR should not be applied to food and water directly intended for human consumption. Dispersal using airborne vehicles, and in protected habitats can require specific authorisations.

For details of the uses authorised by the Reference Member State, please see the decision in separate documents.

1.5.3 Information on active substance(s)[2]

Active substance chemical name:	*Bti* Strain AM65-52
Taxonomic names:	Species: *thuringiensis*
	Subspecies: *israelensis*
	Serotype: H-14
	Strain: AM65-52
	Genus: *Bacillus*
	Family: Bacillaceace
CAS No:	n/a
EC No:	n/a

[2] Please insert additional columns as necessary

Purity (minimum, g/kg or g/l):	Concentration of active bacteria is at average 28 g/kg product or $1.3*10^{12}$ CFU/kg or $1.9*10^{8}$ ITU/kg. Information concerning impurities and additives is confidential to Valent BioSciences and is presented in the confidential attachment under Point IIIA 1.4.2.1 and IIIA 1.4.2.2 in the application.
Inclusion directive:	Directive 2011/78/EU
Date of inclusion:	2013-10-01
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:	The addresses of the manufacturer and of the location of the manufacturing sites of the active substance and the product were the same as when the active substance was evaluated by IT at EU level. Information can be found in the confidential part of the Assessment report on *Bacillus thuringiensis* subsp. *israelensis* Serotype H-14 Strain AM65-52 (according to Directive 98/8/EC).

1.6 IDENTITY RELATED ISSUES

1.6.1 Information on the substance(s) of concern[3]

The products contain no substances of concern.

1.7 DOCUMENTATION

1.7.1 Data submitted in relation to product application

New studies concerning VectoBac G and VectoBac GR have been submitted and are listed in annex 1. Most of these studies are connected to the physical properties and to the efficacy of VectoBac G and VectoBac GR. References taken from the open literature used in the evaluation can be found in a separate list in annex 1.

1.7.2 Access to documentation

The applicant, Sumitomo Chemical Agro Europe SAS, owns the data on the active substance *Bacillus thuringiensis* subspecies *israelensis* serotype H-14, strain AM65-52 supporting this product authorisation, therefore there is no need for a Letter of Access. The applicant was also the notifying company for Annex I inclusion of the active substance.

[3] Please insert additional columns as necessary

2 SUMMARY OF THE PRODUCT ASSESSMENT

Ref-MS Information to the reader:	The following section (Section 2) of the Product Assessment Report for the biocidal products VectoBac G and VectoBac GR is based on the applicant's text and tables from submitted Documents IIB, IIC, and a few tables summarizing specific studies. The format of the documents, such as section and table numbering or the layout, has been altered to conform to the formatting of this Product Assessment Report. For example, Section 1 in document IIC is Section 2.7 in this Product Assessment Report. Also, Sections 3 and 4 in document IIC have been removed as the text is mostly identical to Sections 5.4 and 6 in document IIB (sections 2.5.4 and 2.6 in this document). Apart from this, the contents of this section have not been amended by the Ref-MS, unless otherwise stated (see below). Non-professional use is not included in the application for authorisation of this product. The Ref-MS's comments, clarifications, and conclusions are presented in shaded tables or boxes like this one, inserted in the document where considered relevant. In some cases, the applicant´s text has been shaded and marked with asterisks (*) referring to the adjacent Ref-MS's commenting box. For the review of the application, the Ref-MS has focused on the elements which are crucial for risk assessment and decision-making; hence, minor errors or discrepancies from the view of the Ref-MS of no importance for the overall conclusion, or the specific phrasing of the text, are not amended or commented upon.

2.1 GENERAL PRODUCT INFORMATION

2.1.1 Identification of the biocidal product

Data presented in this product dossier have been derived from two similar versions of the granule product; 'VectoBac' GR and 'VectoBac' G. The two products are very similar in their construction and composition. They both contain the same amount of active substance and carrier absorbed onto a natural granular support. It is therefore reasonable to consider that the toxicological and ecotoxicological characteristics of the two products will be similar. Therefore, throughout this dossier, wherever 'VectoBac' G is stated, reference to 'VectoBac' GR is also inferred.

'VectoBac' WG was the representative product supporting Annex I inclusion of *Bti* Strain AM65-52. 'VectoBac' G and 'VectoBac' GR are similar products (being granules rather than water-dispersible granules) that contain the same active microorganism.

Ref-MS information to the reader:	The two different formulations are considered equal in all aspects essential for the product approval. Also there is no indication that specific activities, health risks or environmental concerns related to the products are in any way different to what has been observed for the active microorganism or the representative product VectoBac WG. Any potential risk connected to VectoBac WG is valid for VectoBac G and VectoBac GR. Unless otherwise stated, in all "Ref-MS information boxes", the term VectoBac could be read as either VectoBac G or VectoBac GR. To conclude we agree that the two products can be evaluated simultaneously and the endpoints given will be accepted throughout this report.

2.1.1 Identity of ingredients of the biocidal product

Information on the composition of 'VectoBac' G is confidential to Valent BioSciences and is presented in the confidential attachment under Point IIIB 1.4.

Ref-MS information to the reader:	In the confidential attachment, routine procedures to confirm absence of microbial contaminants are presented. The biopotency of each batch is estimated using mosquito larva at lab scale level using a protocol described in Annex 2 of this document.

2.1.2 Physico-chemical properties

'VectoBac' G consists of nearly dust free, physically stable, pale brown granules. 'VectoBac' GR is similarly a granule product. Based on 'VectoBac' G data, the formulations have the desirable technical characteristics of a granule. During storage in commercial packaging over 24 months, the potency of the active ingredient remains acceptable and the technical characteristics remain effectively unchanged. In terms of their physical performance, 'VectoBac' G and 'VectoBac' GR are considered to be good and acceptable products. 'VectoBac' G and 'VectoBac' GR are not highly flammable and do not have oxidising or explosive properties. The products do not possess any other physico-chemical properties that indicate a particular physical hazard during storage, transport or use.

Table 2.1.2-1 Physico-chemical properties of VectoBac G and VectoBac GR

Table 2.1.2-1 Physico-chemical 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)	Visual inspection	VBC-60241, lot 185-641-N800 a.i. content not specified	Pale brown granules	Y	IIIB 2.1/01 Comb, A. (2013)
	Visual inspection	VBC-60233, lot 92-042-VB, 92-043-VB, 92-044-VB, 92-045-VB a.i. content not specified	Granules	Y	IIIB 2.1/02 Harding, L.. (2010)
IIIB 2.2					
IIIB 2.2.1 Effects of light, temperature and humidity on technical characteristics of the biocidal product	The product was stored for 24 months at 20°C and 25° in the commercial container	VBC-60241, lot 185-641-N800 a.i. content not specified	The measured mean potency expressed as a % of a frozen control sample was as follows after 24 months storage: 20°C storage 104% (194 ITU/mg) 25°C storage 99.5% (185 ITU/mg) The results for technical properties are given in the relevant sections below. There were no significant differences in the physical properties of VBC-60241 following storage, indicating physical stability of the formulation. Consequently it can be concluded that the test substance was stable when stored in the commercial container for a period of 24 months at 20°C and 25°C.	Y	IIIB 2.2/01 Comb, A. (2013)
IIIB 2.2.2 Other factors affecting stability	No other information regarding stability is required or has been submitted.				
IIIB 2.3 Explosivity and	EC method A14 explosive properties	Not relevant	It can be concluded that 'VectoBac' G is unlikely to undergo	N	IIIB 2.3/01 Curl, M.G.,

oxidising properties	Theoretical assessment		rapid decomposition with the evolution of gases or release of heat and does not therefore present a risk of explosion.		(2013a)
	EC method A14 explosive properties Theoretical assessment	Not relevant	It can be concluded that 'VectoBac' GR 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 2.3/02 Curl, M.G., (2013d)
	EC method A17 oxidising properties Theoretical assessment	Not relevant	It can be concluded that 'VectoBac' G will not be an oxidizer and will be capable of reacting exothermically with combustible materials.	N	IIIB 2.3/03 Curl, M.G., (2013b)
	EC method A17 oxidising properties Theoretical assessment	Not relevant	It can be concluded that 'VectoBac' GR will not be an oxidizer and will be capable of reacting exothermically with combustible materials.	N	IIIB 2.3/04 Curl, M.G., (2013e)
	Information in these reports is confidential to Valent BioSciences and is presented in the confidential attachment under Point IIIB 2.3-01 - Point IIIB 2.3-04.				
IIIB 2.4 Flashpoint and other indications of flammability or spontaneous ignition	'VectoBac' G is not a liquid formulation and therefore flash point is not relevant. The relative self ignition test has not been conducted on 'VectoBac' G. This test is not relevant to granular formulations as it is not used for classification purposes. Theoretical assessment of flammability: It can be concluded that 'VectoBac' G will not be classified as highly flammable. (Curl, M.G., 2013c). Information in this report is confidential to Valent BioSciences and is presented in the confidential attachment under Point IIIB 2.4-01.				
	'VectoBac' GR is not a liquid formulation and therefore flash point is not relevant. The relative self ignition test has not been conducted on 'VectoBac' GR. This test is not relevant to granular formulations as it is not used for classification purposes. Theoretical assessment of flammability: It can be concluded that 'VectoBac' GR will not be classified as highly flammable. (Curl, M.G., 2013f). Information in this report is confidential to Valent BioSciences and is presented in the confidential attachment under Point IIIB 2.4-02.				
IIIB 2.5 Acidity, alkalinity and pH value	CIPAC MT75.3	VBC-60241, lot 185-641-N800 a.i. content not specified	pH = 5.1 and following 24 months storage at: 20°C: pH = 5.8 25°C: pH = 5.8	Y	IIIB 2.5/01 Comb, A. (2013)
	CIPAC MT75.3	VBC-60233, lot 92-042-VB, 92-043-VB, 92-044-VB, 92-045-VB a.i. content not specified	pH = 5.41, 5.21, 5.15, 5.18	Y	IIIB 2.5/02 Harding, L.. (2010)

IIIB 2.6 Viscosity and surface tension	'VectoBac' G and 'VectoBac' GR are not a liquid formulations and therefore viscosity and surface tension are not relevant.				
IIIB 2.7	**Technical characteristics**				
IIIB 2.7.1 Wettability	'VectoBac' G and 'VectoBac' GR are ready to use granular formulations that are to be applied directly without dilution. 'VectoBac' G is not a wettable granule and therefore wettability is not relevant.				
IIIB 2.7.2 Persistent foaming	'VectoBac' G and 'VectoBac' GR are ready to use granular formulations that are to be applied directly without dilution, therefore, persistence of foaming is not relevant.				
IIIB 2.7.3 Suspensibility and suspension stability	'VectoBac' G and 'VectoBac' GR are ready to use granular formulations that are to be applied directly without dilution, therefore, suspensibility and suspension stability are not relevant.				
IIIB 2.7.4 Wet sieve and dry sieve test	'VectoBac' G and 'VectoBac' GR are ready to use granular formulations that are to be applied directly without dilution. 'VectoBac' G is not a dustable powder and therefore a dry sieve test to determine if the particle size is suitable is not relevant.				
	'VectoBac' G and 'VectoBac' GR are ready to use granular formulations that are to be applied directly without dilution therefore a wet sieve test is not relevant.				
IIIB 2.7.5 Particle size distribution, content of dust/fines, attrition and friability	CIPAC MT58.2 Particle size distribution	VBC-60241, lot 185-641-N800 a.i. content not specified	Size range: 99.6% greater than 850µm and following 24 months storage at: <u>20°C storage</u> 99.5% greater than 850µm <u>25°C storage</u> 99.8% greater than 850µm	Y	IIIB 2.7.5/01 Comb, A. (2013)
	CIPAC MT171 Dustiness	VBC-60241, lot 185-641-N800 a.i. content not specified	Collected dust: 0.03mg and following 24 months storage at: <u>20°C storage</u> 0.07mg <u>25°C storage</u> 0.03mg The test material is "nearly dust-free" and remained "nearly dust-free"after storage.	Y	IIIB 2.7.5/01 Comb, A. (2013)
	CIPAC MT171 Dustiness	ABG-6189, lot 185-26A-N8 a.i. content not specified	Collected dust: 0.05mg The test material is "nearly dust-free".	Y	IIIB 2.7.5/02 Woolley, A and Mullee, D. (2004)
	CIPAC MT171 Dustiness	VBC-60233, lot 92-042-VB, 92-043-VB, 92-044-VB, 92-045-VB a.i. content not specified	Collected dust: <1.0mg The test material is "nearly dust-free" and remained "nearly dust-free" after storage.	Y	IIIB 2.7.5/03 Harding, L.. (2010)
	CIPAC MT178 Attrition resistance	VBC-60241, lot 185-641-N800	Attrition resistance: 99.7%	Y	IIIB 2.7.5/01

		a.i. content not specified	and following 24 months storage at: 20°C: 99.7% 25°C: 99.9%		Comb, A. (2013)
	CIPAC MT178 Friability and attrition	ABG-6189, lot 185-26A-N8 a.i. content not specified	Attrition resistance: 99.8%	Y	IIIB 2.7.5/02 Woolley, A and Mullee, D. (2004)
	CIPAC MT178.2 Friabile attrition	VBC-60233, lot 92-042-VB, 92-043-VB, 92-044-VB, 92-045-VB a.i. content not specified	Attrition resistance: 99.82%, 99.85%, 99.85%, 99.81%	Y	IIIB 2.7.5/03 Harding, L. (2010)
jIIIB 2.7.6 Emulsifiability, re-emulsifiability, emulsion stability	'VectoBac' G and 'VectoBac' GR are ready to use granular formulations that are to be applied directly without dilution. 'VectoBac' G and 'VectoBac' GR are not emulsifiable formulations and therefore emulsion characteristics are not relevant.				
IIIB 2.7.7 Flowability, pourability and dustability	According to the ECHA guidance on information requirements for biocidal products (V1.0, July 2013) the flowability test is only required for granular formulations that are applied through equipment that would subject the granules to pressure and/or heat. 'VectoBac' G and 'VectoBac' GR are applied manually and not subject to pressure and/or heat. The products have been used for many years and there has been no incidence of the product loosing flowability on storage.				
IIIB 2.8	**Physical, chemical and biological compatibility with other products**				
IIIB 2.8.1 Physical compatibility	'VectoBac' G and 'VectoBac' GR are not intended for application as tank mixed formulations and therefore information regarding compatibility with other products is not relevant.				
IIIB 2.8.2 Chemical compatibility	'VectoBac' G and 'VectoBac' GR are not intended for application as tank mixed formulations and therefore information regarding compatibility with other products is not relevant.				
IIIB 2.8.3 Biological compatibility	'VectoBac' G and 'VectoBac' GR are not intended for application as tank mixed formulations and therefore information regarding compatibility with other products is not relevant.				
IIIB 2.9 Summary and evaluation of physical, chemical and technical properties	'VectoBac' G consists of nearly dust free, physically stable, pale brown granules. The formulation has the desirable technical characteristics of a granule. During storage in commercial packaging over 24 months, the potency of the active ingredient remains acceptable and the technical characteristics remain effectively unchanged. 'VectoBac' GR consists of nearly dust free granules. The formulation has the desirable technical characteristics of a granule. In terms of its physical performance, 'VectoBac' G and 'VectoBac' GR are considered to be good and acceptable products. 'VectoBac' G and 'VectoBac' GR are not highly flammable and do not have oxidising or explosive properties. They do not possess any other physico-chemical properties that indicate a particular physical hazard during storage, transport or use.				
IIIB 2.10 Other: Bulk density	CIPAC MT186	VBC-60233, lot 92-042-VB, 92-043-VB, 92-044-VB, 92-045-VB a.i. content not specified	Tap Density: 0.75g/ml, 0.74g/ml, 0.75g/ml, 0.75g/ml Pour Density: 0.67g/ml, 0.67g /ml, 0.67g /ml, 0.67g /ml	Y	IIIB 2.10/01 Harding, L.. (2010)

Ref-MS information to the reader:	The data in Table 2.1.2-1 are directly taken from file IIIB 2Phys-chem G-GR. Conclusion: VectoBac' GR and G are not expected to be explosive, oxidizing or highly flammable. Test results for relevant technical characteristics of VectoBac' GR and G satisfies the requirements of granules. VectoBac' GR and G show physical stability and compatibility with their proposed commercial packaging after storage for two year storage at 20°C and 25°C. From a low level, observed bacterial contaminants are slightly increased after 24 months storage. However, this change may not be significant and as *Bacillus* spores are, in comparison to any bacterial vegetative cell, extremely tolerant, no specific caution has to be taken to monitor accumulation of contaminants during storage. Also, when screening for common pathogens, no colonies could be detected. The screened pathogens,*Escherichia coli*, *Pseudomonas sp.*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, were all selected on standard species (genus) selective media. The product efficiency remains intact after 24 months storage. Notable, is that the efficiency is measured only as ITU not CFU. Hence, it is possible that the spore viability is reduced but the amount of toxic crystals is intact.

2.1.3 Analytical methods for detection and identification

Information regarding analytical methods for the detection and identification of the active microorganism are provided by the studies evaluated and concluded in the Annex I Assessment Report for *Bti* Strain AM65-52 (February 2010). The methods, based on unambiguous identification at strain level, were considered appropriate for the determination of *Bti* Strain AM65-52 in the formulated product 'VectoBac' G and are shown for completeness in the confidential attachment under Points IIIA 1.3, 1.4, 3.4 and 4.1.

The existing data are considered appropriate for the authorisation of 'VectoBac' G and 'VectoBac' GR, and further data should not be necessary.

Ref-MS information to the reader:	Swedish Chemicals Agency (hereafter KemI) competent authority in Ref-MS does not agree with the statement that the *Bti* Strain AM65-52 can be unambiguously identified at the strain level as in all their methodology other *Bti* are not included. However, KemI agrees that the method is considered appropriate for the determination of Bti strain AM65-52 in the formulated product. Hence, it is possible to discriminate Bti from any potential contaminant, e.g. *Bacilli* pathogens, including *B. cereus* and *B. anthracis*, during manufacturing. For product approval the supplied methods for strain/species discrimination are acceptable. A method enabling discriminating the particular strain from other *Bti* would have been optimal but possibly not achievable. Consequently, there is no reliable method to distinguish *Bti* Strain AM65-52 to other *Bti* strains from collected field samples.

2.1.4 Classification and labelling

'VectoBac' G does not possess any physico-chemical properties that indicate a particular physical hazard during storage, transport or use and therefore the product is not classified under Regulation (EC) No. 1272/2008 on the basis of its physico-chemical properties.

'VectoBac' G poses no quantifiable risk to human health or the environment and does not require classification for acute toxicity, irritation/corrosivity, sensitising properties, or for environmental hazard according to Regulation (EC) No. 1272/2008. Other components in the 'VectoBac' G formulation are similarly not classified.

Consequently, 'VectoBac' G should not require classification for human health effects or environmental hazard under Regulation (EC) No. 1272/2008.

The micro-organism *Bti* Strain AM65-52 is not infective and the use of 'VectoBac' G is unlikely to cause human disease. The product can therefore be classified as a Group 1 biological agent according to Article 2 of Directive 2000/54/EC. The use of the biohazard symbol on the product label is not required. Use of the precautionary statement *"Contains Bacillus thuringiensis, micro-organisms may have a potential to provoke sensitising reactions"* is required.

The product 'VectoBac' G has been shown not to be a sensitiser. Like all microbials, the micro-organism *Bti* Strain AM65-52 is regarded as a potential skin and respiratory sensitiser according to the conclusions presented in the Annex I Assessment Report. As a consequence product labels for 'VectoBac' G require the following phrases:

"*Contains Bacillus thuringiensis, micro-organisms may have a potential to provoke sensitising reactions"*

"*Wear suitable protective clothing (coveralls), suitable protective gloves and suitable respiratory protective equipment when mixing or loading the product*".

Ref-MS information to the reader:	The text in Section 2.1.4 concerning Classification and Labelling is in agreement with the Annex 1 Assessment Report (CAR) and KemI do not oppose to the proposed precautionary statements in principle. However, in section 2.6 the applicant suggests different phrases; S24/25 and S36/37, and in the submitted application form the phrases P262 and P280 from the CLP-regulation are suggested. After consulting the applicant we have agreed on the following precautionary statements from the CLP-regulation: P261: Avoid breathing dust. P280: Wear protective gloves/protective clothing P302 + P352: IF ON SKIN: Wash with plenty of soap and water. P363: Wash contaminated clothing before reuse. P501: Dispose of contents/container in accordance with local regulation. Additional information regarding **packaging of the biocidal product**: Material: Aluminium laminated foil bag in polyester and nylon. Capacity: 18.41 kg (the authorised package size in the SPC will read "Minimum 18 kg").

The above classification and labelling conclusions are valid also for 'VectoBac' GR.

2.2 EFFICACY

2.2.1 Use and product type

'VectoBac' G and 'VectoBac' GR are biological larvicide used as insecticides (PT18) for Pest Control (Main Group 3).

2.2.2 Details of intended use

Field of use Product type	Application	Number and timing	Waiting period
Control of mosquito larvae in water habitats Product Type 18	Ground application as a coarse surface spray.	*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, since during the later part of the 4th instar growth stage the larvae are no longer eating and the product will not be effective.	None

Ref-MS information to the reader:	The table above is considered by KemI as an example as VectoBac may also, in accordance with the product application, also be applied aerially. Also, it is unclear to KemI what the column "Waiting period" actually stands for but we assume it stands for the period between application and when the product is active against its targets.

2.2.3 Application rate

Under normal conditions, 'VectoBac' G and 'VectoBac' GR are applied to mosquito larval habitats at dose rates ranging from 2.5 -15 kg/ha (equivalent to 70 to 420 g *Bti* Strain AM65-52/ha, based on a composition of 2.8% w/w fermentation solid and soluble), depending on the population density and water quality.

Ref-MS information to the reader:	The proposed maximal dose of 15 kg/ha corresponds to $2.0*10^{13}$ CFU/ha or $3*10^{9}$ ITU/ha. After contacting the application we received the following response relating to recommended dosage: " **Low dose 2.5 kg/ha – 10 kg/ha** Relatively clear water in which mosquito larvae proliferate such as irrigation ditches; reservoirs, lakes, rivers, canals, marshland, ponds; catch basins, drainage and roadside ditches; all other natural or manmade aquatic sites or containers in which mosquito larvae are present. **High dose 10 kg/ha – 15 kg/ha** 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; Mosquito larval habitat covered with dense vegetation. All other natural or manmade aquatic sites or containers, whether 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 L4 stages

2.2.4 Conditions under which the product may not be used

'VectoBac' G and 'VectoBac' GR are not intended for use in finished treated drinking water.

2.2.5 Method of application

'VectoBac' G may be applied using conventional ground or aerial application equipment.

2.2.6 Number and timing of applications

The active ingredient is specific to larvae of certain *dipteran* insect species*. Consequently 'VectoBac' G and 'VectoBac' GR are larvicide products and the timing of application will depend on the level of larvae infestation and the growth stage. The products should be applied during the 1st to the early 4th larval instar, since during the later part of the 4th instar growth stage the larvae are no longer eating and the products will not be effective. A maximum number of applications of eight per season is considered.

2.2.7 Proposed instructions for use

Sample labels are available on request.

2.2.8 Efficacy information

Results from 22 efficacy studies have been included and summarised in Document IIIB Section 6.

The trials were carried out in various habitats across Europe and were selected on the basis of a history of mosquito infestation, in areas representative of mosquito habitat, such as standing water and including rice fields. Trial plot sizes ranged from small *ca* 15 L water containers to 5 ha fields. Trials were carried

out by Officially Recognised organisations. The trials assessed the reduction in numbers of mosquito larvae following application of 'VectoBac' G at rates between 2.5 and 22 kg/ha. In each trial there was an untreated control and at least one rate of the test product. Treatments were applied by hand or using standard application equipment. Application of 'VectoBac' G, at rates covering the proposed label rate, resulted in mean percentage larval mortality of between 88.7 and 100.0% indicating the product to be highly effective.

The product 'VectoBac' GR contains the same percentage of active microorganism (*Bti* Strain AM 65-52) as 'VectoBac' G and non-active co-formulants used in the two products are qualitatively and quantitatively very similar. The intended use pattern and target organism (mosquito) for VectoBac' GR and 'VectoBac' G are the same. The available data for 'VectoBac' G is therefore considered to provide suitable evidence that 'VectoBac' GR will also be effective for the control of mosquito larvae and separate efficacy trials with the 'VectoBac' GR are not considered necessary and have not been conducted.

Table 2.2.8-1 Summary of effectiveness.

Trial report GEP Yes/No	Location	Pest	Habitat	Number larvae in control	% Efficacy of VectoBac G (relative to untreated control Kg/ha)	Ground or aerial application (G, A or G&A)
IIIB, 6.1/12 2004PDECH 033 (Trial 1) GEP No	Italy	Mosquito - *O. caspius, C. modestus* and *A. maculipennis*	Rice fields (30 cm high)	12	98.3 (10)	A
IIIB, 6.1/13 2004PDECH 033 (Trial 2) GEP No	Italy	Mosquito - *O. caspius, C. modestus* and *A. maculipennis*	Rice fields (50 cm high)	9	99.4 (10)	A
IIIB, 6.1/04 JAMCA Manuscript VectoBac KABS (Trial 1) GEP No	Poland	Mosquito - *Aedes caspius, A. vexans* and *Culex pipiens pipiens*	Sewage reservoirs and Irrigation fields	9.9 -16.4	93.1 (5) 97.4 (10)	G&A
IIIB, 6.1/01 JAMCA Manuscript VectoBac KABS (Trial 2) GEP No	Germany	Mosquito - *Aedes caspius, A. vexans* and *Culex pipiens pipiens*	Sewage reservoirs and Irrigation fields	13	94.0 (5) 97.0 (10) 93.0 (15)	G&A
IIIB, 6.1/05 2009RFUSC 004 GEP No	France	Mosquito - *Culex* spp.	Water treatment plant water - decanted into barrels	90	100 (5) 100 (10) 100 (15) 100 (20)	G
IIIB, 6.1/06 2009HKOTT 005b GEP No	France	Mosquito - *Aedes rusticus* and *Aedes cantans*	Not stated	218, 89, 75, 12	54.2 (8) 99.9 (10) 84.0 (15) 94.9 (15)	G
IIIB, 6.1/07 2009HKOTT 006 report 1 GEP No	France	Mosquito- *Culex* sp. and *Anopheles* sp.	Natural conditions - ditches	18	100 (4) 100 (7) 100 (8) 100 (12)	G
IIIB, 6.1/08 2009HKOTT 006 report 2 GEP No	France	Mosquito- *Culex* sp. and *Anopheles* sp.	Natural conditions - ditches	1 to 15	100 (8)	G

Trial report GEP Yes/No	Location	Pest	Habitat	Number larvae in control	% Efficacy of VectoBac G (relative to untreated control Kg/ha)	Ground or aerial application (G, A or G&A)
IIIB, 6.1/17 2010HKOTT 009a GEP No	Spain	Mosquito- *Culex* sp.	Rice fields	Not given	100 (4) 100 (7) 100 (9)	G
IIIB, 6.1/18 2010HKOTT 009b GEP No	Spain	Mosquito - *Anopheles* sp.	Rice fields	Not given	100 (4) 100 (7) 100 (9)	G
IIIB, 6.1/19 2010HKOTT 008 - 1 GEP No	Spain	Mosquito - *Anopheles* sp.	Rice fields	103, 130, 72 and 80	100 (4) 100 (8) 100 (12) 100 (16)	G
IIIB, 6.1/20 2010HKOTT 008 - 2 GEP No	Spain	Mosquito- *Culex* sp.	Rice fields	373, 474, 622, 217	90.3 (4) 81.9 (8) 99.8 (12) 100 (16)	A
IIIB, 6.1/02 2010PDECH 012 GEP No	Germany	Mosquito- *Anopheles* sp.	Semi-field (buckets placed in urban area)	Not stated	89 (3) 100 (5) 100 (10)	G
IIIB, 6.1/14 2010PDECH 013 GEP No	Italy	Mosquito- *Culex* sp. and *Anopheles* sp.	Rice fields	Not stated	100 (7)	A
IIIB, 6.1/03 2010PDECH 041 GEP No	Germany	Mosquito- *Aedes* sp.	Woodland pools	180, 275, 255	84.0 (7) 87.0 (10) 97 (15)	G
IIIB, 6.1/09 2012HKOTT 01 GEP No	France	Mosquito- *Aedes* sp. and *Ochlerotatus caspius* and *O. detritus*	Saltmarsh	6 to 16/dip	90.0 (3) 95.0 (6) 100 (9) 100 (12)	G
IIIB, 6.1/10 2012HKOTT 005 report 1 GEP No	France	Mosquito - *Aedes vexans, Ochlerotatus sticticus*	Ditches in forest and fields	290 and 65 and 800	100 (4) 100 (7)	G
IIIB, 6.1/11 2012HKOTT 005 report 2 GEP No	France	Mosquito - *Aedes vexans, Ochlerotatus sticticus*	Ditches in fields	2000 and 380	94.5 (4) 100 (7)	G
IIIB, 6.1/15 2012HKOTT	Italy	Mosquito - *Aedes* sp.	Rice fields	4.65/dip	100 (3) 100 (6)	G

Trial report GEP Yes/No	Location	Pest	Habitat	Number larvae in control	% Efficacy of VectoBac G (relative to untreated control Kg/ha)	Ground or aerial application (G, A or G&A)
010 GEP No					100 (9)	
IIIB, 6.1/16 2012HKOTT 011 GEP No	Italy	Mosquito - *Culex* sp.	Rice fields	up to 9.55/dip	92.0 (3) 100 (6) 100 (9)	G
IIIB, 6.1/22	Sweden	*A. sticticus*	Wet lands	19.0-64.7/dip	91-100 (13) 100 (22)	A

Ref-MS information to the reader:	Table 2.2.8-1 is a modified version of Table IIIB 6.1-02: Summary of effectiveness. Besides indicating the efficiencies, the Table gives an adequate overview of different habitats tested. To the Table, KemI has introduced another submitted study IIIB, 6.1/22. To conclude: Based on submitted studies and several open peer-reviewed articles it is shown that VectoBac G is an effective insecticide, or more specifically a larvicide. Note, the quality of some studies can be questionably and one study is submitted in triplicates (studies numbered 12, 13 and 23 are identical). For some of the studies, the proposed maximal dose of 15 kg/ha had to be applied to obtain an efficacy above 90 % but generally a lower concentration was sufficient to obtain an efficacy of 100 %. In addition, the applicant has attached published peer-review articles that support the efficacy of *Bti*. Under all circumstances, efficacy is dependent on correct use and the product should only be applied by trained personnel. KemI agrees with the statement that no separate studies for VectoBac GR are necessary. *Briefly the toxicity is specific to larval stages of Culicidae (mosquitos), Simuliidae (black flies) and a few other Nematoceran species, e.g. some species in the genus *Chironomus*.

2.2.9 Information on the possible occurrence of the development of resistance

In the laboratory, resistance has been developed for several insects to the *Bacillus thuringiensis* subspecies *kurstaki*, *aizawai*, *entomocidus* and *tenebrionis* (*san diego*) and individual Cry toxins from the subspecies *kurstaki*, *aizawai*, *entomocidus* and *israelensis*. However, despite repeated attempts, significant resistance to whole cultures of *Bti* has not been achieved. The difficulty of generating resistance results from the involvement of Cyt toxins, which appear capable of overcoming resistance generated to individual or multiple Cry toxins. No established resistance to Bti has been described in the field, even after years of use. The simultaneous production of six different toxins by *Bacillus thuringiensis israelensis* (Bti) is thought to delay the evolution of resistance in treated mosquito populations. Cross resistance between *Bacillus thuringiensis* toxins has been found, but does not automatically occur. In most cases, with the exception of *Bti*, resistance to *Bacillus thuringiensis* was developed in the laboratory in less than 20 generations.

Based on this information, the Annex I Assessment Report for *Bti* Strain AM65-52 (February 2010) concluded no concerns for the development of resistance to the active microorganism.

Ref-MS information to the reader:	KemI agrees with the statements concerning resistance development: In open literature, no resistance to *Bti* has been observed in the field. Partial resistance to *Bti* has been reported in laboratory trials after 20 generations, with an increased sensitivity recorded three generations after the treatment was ended. The overall conclusion is that development of resistance in mosquitos is unlikely, and under proposed usages the product can, in this respect, be considered as safe.

2.2.10 Effects on the quality of materials or products treated

The Annex I Assessment Report for *Bti* Strain AM65-52 (February 2010) concluded the following effects on the target organism:

'The mode of action of Bti AM65-52 results from toxic proteins contained in parasporal crystals. The crystals are taken up via ingestion and under the alkali conditions present in the larvae gut the crystal dissolves releasing the active protein delta endotoxins (Cry4Aa1, Cry4Ba1, Cry10Aa1, Cry11Aa1 and Cyt1Aa1) that induce disintegration of the larvae gut epithelium and consequent death of the larvae. It is very likely that the death of the insect require septicaemia caused by midgut bacteria'. … There are no other active metabolites and degradation products that are known to contribute to the toxicity of Bti (Strain AM65-52)'.

'VectoBac' G and 'VectoBac' GR are used for the control of mosquito in water habitats. Due to the specific mode of action, the products will have no other effects on the intended area of use.

Ref-MS information to the reader:	KemI agrees with the specific mode of action as it is well documented and adequately described in the CAR as well as in open literature. Noticeable, is the fact that the toxic crystals became active under alkaline conditions within the larval gut. At low pH, e.g. in the mammalian stomach, the crystals are non-toxic. The question in the risk assessment is rather whether populations of related insects can be unacceptably reduced after treatment with *Bti*. Other non-target effects, if present, are most likely dependent on modes of action not connected to the larvicidal crystals.

2.3 EXPOSURE ASSESSMENT

2.3.1 Intended uses

'VectoBac' G and 'VectoBac' GR are used for the control of mosquito in water habitats. Details are shown in Section IIB 2.2.* The products are used strictly by professional operators only within Europe, treating waters where mosquito larvae are breeding.

Ref-MS information to the reader:	* Section 2.2.2 in this document. However, examples of water habitats are best given in Table 2.2.8-1.

2.3.1.1 Human exposure assessment

The uses supported in this dossier are essentially the same uses detailed for the representative product that was evaluated and concluded in the Annex I Assessment Report for *Bti* Strain AM65-52 (February 2010). The active substance in the product VectoBac G is the same as in the Annex I Assessment Report for *Bti* Strain AM65-52 (February 2010). The information presented below in Sections 3.2.1 to 3 2 4 is taken from the Annex I Assessment Report and is included for completeness.

The existing exposure assessments (completed for VectoBac WG) are considered appropriate for the authorisation of 'VectoBac' G and further assessment should not be necessary.

2.3.1.2 Identification of main paths of human exposure to the active substance from its use in the biocidal product

Inhalation exposure

'VectoBac' G is a granular (G) formulation. Users could be exposed by inhalation during mixing/loading of the granules. However, the formulation is non-dusty, which will reduce the potential for inhalation exposure during mixing/loading, and only professionals apply the product and professional users are required to wear a dust/mist filtering respirator to reduce inhalation exposure during mixing/loading and during application if not in enclosed tractor cabs. Only professional users wearing protective equipment are permitted in areas being treated.

Dermal exposure

'VectoBac' G is a granular formulation. Users could be exposed dermally during mixing/loading of the granules and during application. However, the formulation is a granule which will reduce the potential for dermal exposure of the hands during mixing/loading as the particles will not adhere to gloved hands. Users are required to wear long-sleeved shirt, long trousers, shoes and socks, and water-proof gloves to reduce dermal exposure during mixing/loading and application. Only professional users wearing protective equipment are permitted in areas being treated.

Oral exposure

'VectoBac' G is not likely to reach the mouth of professional users. Professional users are required to wear a dust/mist filtering respirator to reduce oral exposure during mixing/loading and during application if not in enclosed tractor cabs.

2.3.1.3 Professional exposure

Bti Strain AM65-52 has been shown through maximum challenge protocols and innocuity, infectivity and pathogenicity tests to have no adverse effects on human health. On this basis it is possible to exclude the probability of toxic effects of the product on exposed operators or workers. Professionals apply VectoBac G directly to the surface of casual waters by spraying or by use of pumps to treat filter beds in STPs. Casual waters are sprayed as required dependent on the development stages of the mosquito larvae present. Use of tractors with closed cabs is recommended for spray applications and in all other scenarios the use of respiratory protection and gloves is required PPE.

Therefore the professional use of VectoBac' G is considered safe with the following limits:

Professional users are required to wear PPE. In detail: long-sleeved shirt, long trousers, shoes and socks, and water-proof gloves to reduce dermal exposure during mixing/loading and application, and a dust/mist filtering respirator to reduce inhalation exposure during mixing/loading and during application if not in enclosed tractor cabs. Only professional users wearing protective equipment are permitted in areas being treated.

VectoBac' G 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.

2.3.1.4 Non-professional exposure

There are no non-professional uses of 'VectoBac' G in Europe.

Bystanders whenever possible are excluded from treated areas to ensure only protected professionals can possibly be exposed to 'VectoBac' G

2.3.1.5 Indirect exposure

Bti Strain AM65-52 poses minimal risk to human health and the risks to non-users through indirect exposure are negligible if the biocidal product 'VectoBac' G is used as instructed.

Ref-MS information to the reader:	KemI agrees with the statements above that the risk when handling the active substance is low if all the proposed precautions are followed. In the text, the applicant does not mention the use of airborne vehicles for dispersal of VectoBac. However, KemI cannot see any additional risk as long as the proposed safety requirements connected to aerial application are followed.

2.3.2 Environmental exposure assessment

2.3.2.1 Fate and distribution in the environment

'Vectobac' G is applied to control mosquito larvae in waters where mosquito breeding may occur. It is applied to waters at an application rate of 2.5- 15 kg/ha depending on the population density and quality of the water.

The type of formulation and inert substances used in 'VectoBac' G are not expected to affect the behaviour of the active micro-organism in the environment. None of the formulation components or properties of the formulation are expected to significantly influence the fate and distribution of *Bti* (AM65-52) in the environment. Consequently, the risk assessment has been conducted based on the same principles as the risk assessment for 'VectoBac' WG in the Annex I CA report. As the application rate and concentration of active substance differs between 'VectoBac' G and 'VectoBac' WG, all calculations have been repeated with the appropriate values for 'VectoBac' G.

Degradation of *Bti* Strain AM65-52 vegetative cells and insecticidal toxins in soil (DT_{50} = 5.2 days) and poor germination of *Bti* Strain AM65-52 spores in soil (DT_{50} = 120 days) show that the organism can be fairly persistent but at reduced levels and would poorly multiply in the soil environment. Although *Bacillus thuringiensis* bacteria generally constitute an indigenous part of the soil micro-flora community, they do not compete aggressively with other soil micro-organisms and are fairly adapted to survive as an active member of the soil microbial community. 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.

In water, contact of *Bti* with soil particles resulted in a fast cessation of larvicidal activity (DT_{50} = 14 days) but has no discernible effect on the number of viable bacteria. Disappearance of larvicidal activity is attributed to adsorption of the insecticidal toxins and vegetative cells to soil particles with rapid and virtually complete adsorption of the bacteria onto soil particles. As a realistic worst case, a values of K_{OC} = 1000 can be assumed for adsorption. However, adsorption was reversible with mechanical stirring. Soil adsorbed spores remain viable but do not readily germinate and multiply (DT_{50} = 50 days).* In systems containing only water, inhibition of larvicidal activity was slow but was irreversible showing a gradual degradation of the insecticidal toxins.

The predicted environmental population densities (EEDs) of the active micro-organism and the predicted environmental concentrations (PECs) of toxins are calculated for soil and water based on the worst-case application rate of 15 kg VectoBac G/ha. The maximum number of applications to a single site is 8 with a minimum interval between applications of 7 days. It is assumed that no interception occurs during application.

Ref-MS information to the reader:	KemI agrees with the statements that the formulations do not significantly influence the fate of *Bti* in the environment. KemI also consider the assumptions in the text concerning fate and distribution, based on our knowledge in *Bacillus* physiology, to be adequate and realistic. *The listed endpoints are in line with published endpoints in the Assessment Report. However, in that document as well as in the Product reports the connecting studies are not well indicated.

2.3.2.2 PEC in soil

The PEC and EED in soil was calculated for 8 applications with an interval of 7 days between applications. It was assumed that 'Vectobac' G was applied directly to soil at the maximum application rate of 15 kg/ha. First order dissipation rates between applications were assumed for both the spores and the toxin. The soil half-lives of *Bt* have been experimentally determined to be in the rage of 100-200 days (Hansen *et al.*, 1996) so for the purposes of the risk assessment an average value of 120 days was used. The half-life for insecticidal activity has been calculated to be 2.7-5.2 days. The worst case value of 5.2 days has been used for the risk assessment. For calculation of the EED the DT_{50} for spores of 120 days has been used, and for calculation of PEC values the DT_{50} for toxic effect was used. The following assumptions were used for the calculation of the EED and PEC:

incorporation into the top 5 cm layer

soil density of 1.5 g/ cm³

no adsorption

plant interception: 0 %.

DT_{50} Spores = 120 d, DT_{50} toxin = 5.2 d

The degradation between applications was calculated by multiplying by the degradation constant

constant $= e^{-\frac{\ln 2}{DT50} \times 7}$

The following results were obtained:

Following 1 application:
EEDs = 2.688×10^4 CFU/g
PECs = 4.0 ITU/g

Following 8 applications:
EEDs = 1.875×10^5 CFU/g
PECs = 6.6 ITU/g

2.3.2.3 PEC in surface water, ground water, sediment and STP

'Vectobac' G may be applied directly to surface waters for the control of mosquito larvae as it is a selective bacterial larvicide. As 'VectoBac'G is applied directly to surface waters it is considered necessary to conduct a risk assessment for the aquatic environment.

A risk assessment for surface water and sediment was first carried out with simple step 1 calculations which consider dilution within the waters. This was followed by step 2 calculations which are considered to produce more accurate estimations of the densities of *Bti* in the aquatic environment resulting from the use of 'VectoBac' G.

A lake water dissipation study was reported for *Btk* (Menon and De Mestrel, 1985), but no specific value for the DT_{50} was given. However, in the Annex I CAR a DT_{50} was calculated from the results of this study by the RMS. It is considered appropriate to use this dissipation DT_{50} for 'Vectobac' G as none of the constituents of the product are likely to significantly alter this value. The dissipation of toxins in water has been studied by Mulla *et al.* (1985), Beehler *et al.* (1991), and Hougard *et al.* (1995), and all reported by Glare and O'Callaghan (2000). The DT_{50} value for the toxins has been reported to be within 1-4 weeks for *Bti*. In the Annex I CAR an average half-life of 14 days was estimated by the RMS and is used here for the decline in biological activity of the toxins of *Bti* strain AM65-52.

Ref-MS information to the reader:	KemI agrees with the statement that the average half-life to be used for the toxins is 14 days. This number is also in line with results published in open literature (cited in the text). Note, with the exception of the review book chapter Glare and O'Callaghan, (2000), the references dealing different DT_{50} values are not attached to the application. I.e., only data presented in the review is evaluated by KemI, not the references within.

Surface water and sediment

The EED of *Bti* Strain AM65-52 spores and toxins were calculated for water based on an application rate of 2.5 L 'VectoBac' G/ha and a maximum of 8 repeat applications at a minimum interval of 7 days. As a worst case, no interception was assumed and first order degradation rates for spores (DT_{50} = 50 days) and toxins (DT_{50} = 7 days)* were assumed between applications. The following assumptions were used for the calculation of the EED and PEC:

Ref-MS information to the reader:	* The accepted DT50 value for the toxins is 14 days, not 7. However, in the following calculations the number is 14, i.e. KemI assumes that this is a typing error. Moreover, in the critical risk assessment for *Daphnia*, only the half-life of the spores is relevant (see Ref-MS comments after sessions 2.2.10 and 2.8.1)

water depth 30 cm

interception 0%

DT_{50} Spores = 50 d, DT_{50} toxin = 7 d

The degradation between applications was calculated by multiplying by the degradation constant

$$\text{constant} = e^{-\frac{\ln 2}{DT50}\times 7}$$

The following results were obtained for surface water:

Following 1 application	Following 8 applications
EED_{SW} =6.7 x 10^6 CFU/L	EED_{SW} =3.9 x 10^7 CFU/L
PEC_{SW} = 1.0 x 10^3 ITU/L	PEC_{SW} = 2.0 x 10^3 ITU/L

A Step 2 calculation was conducted using a simple two-compartment model with degradation occurring exclusively in the water phase and the adsorption in the sediment phase, as outlined in the Annex 1 CAR. Distribution to the sediment was based on the distribution constant (K_{oc}) = 1000 mL g^{-1}. This value was presented in the Annex I CAR as it is considered to represent a worst case for calculations with spores and toxins. The sediment bulk density was assumed to be 1.5 g/cm^3 and the sediment depth was assumed to be 5 cm. The DT_{50} values used in the step 2 calculations were the same as those used in the step 1 calculations.

All step 2 calculations for initial concentrations in water were carried out in EXCEL using the following equations:

$$\frac{CFU_{w,t=0}}{L} = \frac{rate\_MPCP(kg/ha) \times \frac{\%\_MPCA}{100} \times 10^3 \times pop\_density(CFU/gMPCA)}{V_L(L/ha)} \times f_{L/T}$$

$$\frac{ITU_{w,t=0}}{L} = \frac{rate\_MPCP(kg/ha) \times biopotency(ITU/mgMPCP) \times 10^6}{V_L(L/ha)} \times f_{L/T}$$

$$f_{L/T} = \frac{1}{1 + K_{ads}\,\delta\,\frac{L_S}{L_L}}$$

For multiple applications, the population densities (CFU/L) and biopotencies (ITU/L) in water after n+1 repetitions (with and interval of 7 days between applications) were obtained using the equations:

$CFU_{init\text{-}w,\ n+1}/L = CFU_{final\text{-}w,\ n}/L + CFU_{w,t=0}/L$

$ITU_{init\text{-}w,\ n+1}/L = ITU_{final\text{-}w,\ n}/L + ITU_{w,t=0}/L$

where:

$CFU_{final\text{-}w,\ n}/L$ = $CFU_{init\text{-}w,\ n}/L$ x dissipation constant

$ITU_{final\text{-}w,\ n}/L$ = $ITU_{initl\text{-}w,\ n}/L$ x dissipation constant

The dissipation constant used was:

constant $= e^{-\frac{\ln 2}{DT50}\times 7}$

The population densities (CFU/g) and biopotencies (ITU/g)) in sediments after n+1 applications were calculated using the following equations:

$CFU_{init\text{-}s,\ n+1}/g = CFU_{final\text{-}s,\ n}/g + CFU_{s,\ t=0}/g$

$ITU_{init\_s,\ n+1} = ITU_{final\text{-}s,\ n} + ITU_{s,\ t=0}$

where:

$CFU_{final\text{-}s,\ n} = CFU_{final\text{-}w,\ n} \times K_{ads}/10^3$

$ITU_{final\text{-}s,\ n} = ITU_{final\text{-}w,\ n} \times K_{ads}/10^3$

and:

$CFU_{s,\ t=0} = CFU_{w,\ t=0} \times K_{ads}/10^3$

$ITU_{s,\ t=0} = CFU_{w,\ t=0} \times K_{ads}/10^3$

The symbols and units used in the equations are as follows:

Symbol	Description	Unit
rate_MPCP	Dose of MPCP added to water	kg/ha
%_MPCA	Percentage of MPCA in MPCP	%
pop_density	*Bti* AM65-52 population density	CFU/g
biopotency	*Bti* AM65-52 biopotency	ITU/mg
CFU	Colony Forming Unit	
$CFU_{init\text{-}w,\ n+1}$	*Bti* AM65-52 population density in water following n+1 applications	CFU/L
$CFU_{init\text{-}w,\ n}$	*Bti* AM65-52 population density in water following n applications	CFU/L
$CFU_{final\text{-}w,\ n}$	*Bti* AM65-52 population density in water after 7 days following n	CFU/L

	applications	
$CFU_{w,t=0}$	*Bti* AM65-52 initial population density in water	CFU/L
$CFU_{init\text{-}s,\ n+1}$	*Bti* AM65-52 population density in sediments following n+1 applications	CFU/g
$CFU_{init\text{-}s,\ n}$	*Bti* AM65-52 population density in sediments following n applications	CFU/g
$CFU_{final\text{-}s,\ n}$	*Bti* AM65-52 population density in sediments after 7 days following n applications	CFU/g
$CFU_{s,t=0}$	*Bti* AM65-52 initial population density in sediments	CFU/g
ITU	International Toxic Units	
$ITU_{init\text{-}w,\ n+1}$	*Bti* AM65-52 biopotency in water following n+1 applications	ITU/L
$ITU_{init\text{-}w,\ n}$	*Bti* AM65-52 biopotency in water following n applications	ITU/L
$ITU_{final\text{-}w,\ n}$	*Bti* AM65-52 biopotency in water after 7 days following n applications	ITU/L
$ITU_{w,t=0}$	*Bti* AM65-52 initial biopotency in water	ITU/L
$ITU_{init\text{-}s,\ n+1}$	*Bti* AM65-52 biopotency in sediments following n+1 applications	ITU/g
$ITU_{init\text{-}s,\ n}$	*Bti* AM65-52 biopotency in sediments following n applications	ITU/g
$ITU_{final\text{-}s,\ n}$	*Bti* AM65-52 biopotency in sediments after 7 days following n applications	ITU/g
$ITU_{s,t=0}$	*Bti* AM65-52 initial biopotency in sediments	ITU/g
$K_{ads}/10^3$	adsorption constant (Freundlich isotherm with exponential term=1)	$L\ g^{-1}$

Inputs and defaults which were used in the two-compartment model are presented below in Table 2.3.3.3-1.

Table 2.3.3.3-1: Inputs and defaults for step 2 water and sediment calculations

Data	Input
Application number	8
Interval between applications (days)	7
Application rate (L/ha)	15
MPCA (%)	2.8
CFU content (CFU/g MPCA)	$4.8x10^{10}$
ITU content (ITU/ mg MPCP)	200
Sediment density (kg/m^3)	1500
Sediment thickness (m)	0.05

Data	Input
Sediment area (m^2/ha)	10000
Sediment volume (m^3/ha)	500
Sediment weight (kg/ha)	750000
Water depth (m)	0.3
Water volume (L/ha)	3000000
Sediment organic carbon (%)	5
Koc (mL/g)	1000
K_D (mL/g)	50
Water $DT_{50,W}$ Spore (days)	50
Water CFU dissipation constant	0.9075
Water $DT_{50,W}$ ITU (days)	14
Water ITU constant	0.7072
$f_{L/T}$ MCPA in water over total	0.0741
$f_{S/T}$ MCPA in sediment over total	0.9259

The results calculated for water and sediment using the step 2 approach are presented below in Table 2.3.3.3-2 to Table 2.3.3.3-7

Table 2.3.3.3-2: Dissipation kinetics of *Bti* AM65-52 in water following single application of 'Vectobac' G to surface water

Time (day)	Concentration (mg/L)	ITU/L	CFU/L
0	0.0104	74.1	497952
1	0.0102	70.52061	491096
5	9.68×10^{-3}	57.85055	464605
10	9.03×10^{-3}	45.16446	433492
20	7.86×10^{-3}	27.52804	377377
30	6.84×10^{-3}	16.77853	328525
40	5.96×10^{-3}	10.22663	285998
50	5.19×10^{-3}	6.2332	248976

Time (day)	Concentration (mg/L)	ITU/L	CFU/L
75	3.67 x10^{-3}	1.80783	176052
100	2.56 x10^{-3}	0.524329	124488
120	1.97 x10^{-3}	0.194788	94344
150	1.29 x10^{-3}	0.044106	62244
200	6.48 x10^{-4}	3.71 x10^{-3}	31122

Table 2.3.3.3-3: Amounts of *Bti* AM65-52 in water following multiple applications of 'Vectobac' G to surface water

Application number	Concentration (mg/L)	ITU/L	CFU/L
1	0.0104	74.1	497952
2	0.0198	126.5035	949843
3	0.0283	163.5633	1359935
4	0.0361	189.772	1732093
5	0.0431	208.3067	2069826
6	0.0495	221.4145	2376319
7	0.0553	230.6843	2654462
8	0.0606	237.24	2906876

The results presented in below in Table 2.3.3.3-4 represent the dissipation of *Bti* AM65-52 in water following 8 applications of 'Vectobac' G, with an interval of 7 days between applications. The dissipation kinetics were calculated using the dissipation DT_{50} values for spores and biopotency where appropriate.

Table 2.3.3.3-4: Dissipation kinetics of *Bti* AM65-52 in water following 8 applications of 'Vectobac' G

Time (day)	Concentration (mg/L)	ITU/L	CFU/L
0	0.0606	237.24	2906876
1	0.0597	225.78	2866865
5	0.0565	185.215	2712251
10	0.0527	144.5987	2530657
20	0.0459	88.13345	2203130

Time (day)	Concentration (mg/L)	ITU/L	CFU/L
30	0.0399	53.71768	1917992
40	0.0348	32.74113	1669759
50	0.0303	19.95585	1453652
75	0.0214	5.787771	1027963
100	0.0151	1.67862	726933
120	0.0115	0.623599	550944
150	7.57×10^{-3}	0.1412	363520
200	3.78×10^{-3}	0.011877	181786

Table 2.3.3.3-5: Dissipation kinetics of *Bti* AM65-52 in sediment following single application of 'Vectobac' G

Time (day)	Concentration (mg/g)	ITU/g	CFU/g
0	5.19×10^{-4}	3.705	24897
1	5.12×10^{-4}	3.526031	24554
5	4.84×10^{-4}	2.892528	23230
10	4.52×10^{-4}	2.258223	21674
20	3.93×10^{-4}	1.376402	18868
30	3.42×10^{-4}	0.838927	16426
40	2.98×10^{-4}	0.511331	24897
50	2.60×10^{-4}	0.31166	12448
75	1.83×10^{-4}	0.090391	8802
100	1.30×10^{-4}	0.026216	6224
120	9.83×10^{-5}	0.009739	4717
150	6.48×10^{-5}	2.21×10^{-3}	3112
200	3.24×10^{-5}	1.86×10^{-3}	1556

Results for sediment presented in terms of wet weight

Table 2.3.3.3-6: Amounts of *Bti* AM65-52 in sediment following multiple applications of 'Vectobac' G

Application number	Concentration (mg/g)	ITU/g	CFU/g
1	5.19 x10^{-4}	3.705	24897
2	9.89 x10^{-4}	6.325176	47492
3	1.42 x10^{-3}	8.178164	67996
4	1.80 x10^{-3}	9.488598	86604
5	2.16 x10^{-3}	10.41534	103491
6	2.48x10^{-3}	11.07073	118816
7	2.77 x10^{-3}	11.53422	132723
8	3.03 x 10^{-3}	11.862	145343

Results for sediment presented in terms of wet weight

The results presented in below in Table 2.3.3.3-4 represent the dissipation of *Bti* AM65-52 in sediment following 8 applications of 'Vectobac' G, with an interval of 7 days between applications. The dissipation kinetics were calculated using the dissipation DT_{50} values for spores and biopotency where appropriate.

Table 2.3.3.3-7: Dissipation kinetics of *Bti* AM65-52 in sediment following 8 applications of 'Vectobac' G

Time (day)	Concentration (mg/g)	ITU/g	CFU/g
0	3.03 x10^{-3}	11.862	145343
1	2.99 x10^{-3}	11.289	143343
5	2.83 x10^{-3}	9.260749	135612
10	2.64 x10^{-3}	7.229934	126532
20	2.29 x10^{-3}	4.406673	110156
30	1.99 x10^{-3}	2.685884	95899
40	1.74 x10^{-3}	1.637057	83487
50	1.51 x10^{-3}	0.997792	72682
75	1.07 x10^{-3}	0.289389	51398
100	7.57 x10^{-4}	0.083931	36346
120	5.74 x10^{-4}	0.03118	27547
150	3.79 x10^{-4}	7.05 x10^{-3}	18176
200	1.89 x10^{-4}	5.94 x10^{-4}	9089

Results for sediment presented in terms of wet weight

The final results following 8 applications of 'Vectobac' G are summarized below in Table 2.3.3.3-8.

Table 2.3.3.3-8: EEDs and PECs for *Bti* AM65-52 in surface water and sediment following 8 applications of 'Vectobac' G

Water			Sediment		
mg MCPA/L	**ITU/L**	**CFU/L**	**mg MCPA/g**	**ITU/g**	**CFU/g**
0.0606	237.24	2906876	3.03×10^{-3}	11.862	145343

Groundwater

Bacillus thuringiensis cells applied to field soils under natural conditions do not move appreciably through the soil profile. 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 the micro-organism *Bti* Strain AM65-52 will not be present in groundwater.

STP

'VectoBac' G may be applied directly to wastewaters in sewage treatment facilities (e.g. effluent lagoons) for the control of mosquito larvae. 'VectoBac' G is applied to sewage treatment plants at the same rate as it is applied to other water bodies, 2.5-15 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. Hence the maximum predicted concentrations of *Bti* in the STP are:

Following 1 application

$EED_{local,\ effluent}$ = 4.9×10^5 [CFU/L]

$PEC_{local,\ effluent}$ = 0.0104 [mg/L]

Following 8 applications

$EED_{local,\ effluent}$ = 2.9×10^6 [CFU/L]

$PEC_{local,\ effluent}$ = 0.0606 [mg/L]

It is likely that these values largely overestimate the density of *Bti* in effluent as sewage treatment plants are designed specifically to remove large amounts of microbial material from the waste stream. Consequently, it is probable that there will be significant removal of *Bti* within the sewage treatment plant and hence the density of *Bti* in the effluent will be much lower than the rate at which it was applied. In addition to this 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.

Any exposure of surface waters from application to sewage treatment facilities in considered to be covered in the surface water assessment as this considers direct application of the product to surface water at the same application rates.

2.3.2.4 PEC in air

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 readily 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. 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.

2.3.2.5 Non compartment specific exposure relevant to the food chain (primary and secondary poisoning)

Two potential routes exist for secondary exposure to *Bti*. Firstly, insect predators may ingest larvae affected by *Bti*, or secondly, dead organic matter may be ingested. However, as the mode of action of *Bti* is so specific, the majority of predators of mosquitos and blackfly will not be affected. 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*) (Section IIIA, 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.

Ref-MS information to the reader:	Sections 2.3.3.2-2.3.3.5: KemI agrees with the calculations indicating low accumulation of *Bti* in soil, groundwater, when used in STP, in air and in exposure relevant to the food chain. In sediment and surface water expected numbers are higher, especially in water after multiple applications. However, with the step 2 calculation when sedimentation of bacteria is taken account the numbers are reduced in water also after 8 applications. The bacterial amount in sediment is also rather low. When assessing the risk to *Bti* the shorter half-life of the toxins is only relevant when the specific mode of action is valid, e.g. against mosquitoes. To non-target organisms only the longer half-life of the spores (120 days) is considered relevant. The hypothetical toxin synthesis of viable bacteria, after being applied to the environment, has been considered, but judged to be insignificant. In *Bti*, synthesis of the toxin crystals is connected to the transformation of vegetative cells to spores, and the survivability of vegetative cells is low. Environmental risk assessments using the calculations above are found in Section 2.8 and discussed there.

2.4 HUMAN HEALTH EFFECTS ASSESSMENT

Information regarding the human health effects of the product 'VectoBac' G is supported by the studies evaluated and concluded in the Annex I Assessment Report for *Bti* Strain AM65-52 (February 2010). Additional study summary information for the product 'VectoBac' G is provided in this dossier in Section IIIB 7 from a battery of acute toxicity investigations with the formulated product, used to conclude the following properties of 'VectoBac' G. The existing data for the active substance and for the in-use product are considered appropriate for the authorisation of 'VectoBac' G and further information should not be necessary.

The oral LD_{50} of 'VectoBac' G, was determined to be greater than 5 x 10^8 cfu in rats and in mice.
The dermal LD_{50} of 'VectoBac' G, was considered to be greater than 5000 mg/kg bw in rats, based on read-across to the active substance data, in the absence of product specific study results to address this endpoint.

The acute inhalation LC_{50} of the test material is greater than the achieved dose level of of 2.04 x 10^6 spores/L (4h) when administered undiluted as an aerosol to albino rats.

'VectoBac' G was not considered to be a skin irritant according to EU classification guidance.

'VectoBac' G is not considered an ocular irritant. None of the mean scores exceeded EU classification requirements.

There was no evidence that 'VectoBac' G induced delayed contact hypersensitivity by dermal contact.

The acute toxicity studies conducted with 'VectoBac' G indicate the product is of low toxicity by the oral and the inhalation routes, and by reference to the active substance, no dermal toxicity is anticipated. The product shows no potential to elicit dermal irritation and causes only limited ocular irritation effects below the threshold for classification. There were no indications the product has the potential to elicit delayed contact hypersensitivity.

Longer term endpoints are derived by read across to studies with the active substance. No evidence for the sub-acute toxicity of *Bti* AM65-52 was found in the dog dosed at *ca* 10^6 *Bti* spores/mL for 90 consecutive days. Rats were exposed for 4 hours a day for 14 consecutive days to an atmosphere containing up to 1.84 x 10^6 spores/L air. There were no mortalities, no treatment-related adverse clinical signs and no changes in the various in-life or post-life parameters that were attributable to treatment with *Bti.*

Standard mutagenicity and genotoxicity assays are not considered appropriate for many living microorganisms nor does the risk they pose often warrant such testing. A waiver request for genotoxicity testing based on testing impracticalities has been presented. Cell culture studies are required for viruses and viroids or specific bacteria and protozoa with intracellular replication. This is not applicable to *B. thuringiensis* which does not replicate in warm-blooded organisms and consequently no cell culture studies are presented for *Bti* AM65-52.

No adverse reactions in individuals as a result of contact with this microbial during its development, manufacture, preparation or filed application have been documented or reported. There have been no medical surveillance abnormalities or reports to the Occupational Health Services from employee at the manufacturing plant to date regarding health related or other adverse reactions. Persistence has been demonstrated in ocular tissue and for organs within the body cavities but without any infectious significance*.

The safety of *Bacillus 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.

Ref-MS information to the reader:	KemI mostly agrees with the conclusions and the risk to human health is considered low. In essence, *Bti* and its crystal toxins are non-toxic to mammals. The applicant has not, in this section, mentioned the distant relationship of *Bt* to harmful Bacilli, i.e. *B. cereus* and *B. anthracis.* However, according to the CAR there is no evidence that *Bti* could lead to infections in humans and we agree with that conclusion. As a precaution, immunocompromised individuals should not be exposed to *Bti*. In infected patients there are methods available to discriminate Bacilli to species level. * This statement, concerning the infectious ability of persistent *Bti*, is not supported by any citation and is, therefore, not considered in the risk assessment.

2.5 ENVIRONMENTAL EFFECTS ASSESSMENT

The type of formulation and inert substances used in 'VectoBac' G are not expected to affect the behaviour of the active micro-organism. Information for the unformulated micro-organism contained in the Annex I Assessment Report can therefore be used to extrapolate to 'VectoBac' G and separate testing of the formulation is not necessary.

The environmental effects of *Bti* Strain AM65-52 concluded in the Annex I Assessment Report are shown in Section 2.5.1 to 2.5.4 for completeness.

2.5.1 Aquatic compartment

Bti Strain AM65-52 is not considered to be acutely toxic to fish; there is no evidence of significant effects with the active microorganism following long-term exposure.

Bti Strain AM65-52 is not considered to be acutely toxic to aquatic invertebrates; there is no evidence of significant effects following long-term exposure, with the exception of influence on offspring production in *Daphnia* (21-day chronic test), where a NOEC = 1 x 10^8 CFU/L (3.3 x 10^3 ITU/L) was observed.

Aquatic plants and algae are not considered to be at risk from *Bti* Strain AM65-52 as there is no mechanism for the ingestion of *Bti* Strain AM65-52 and therefore no appropriate digestive enzymes to enable the release of the active protein δ-endotoxins.

The end-points for the aquatic compartment are summarised in Table IIB 5.1-1

Table 2.5.1-1 – Bti Strain AM6552 - endpoints for the aquatic compartment

Test organism	Test substance	Duration	Effective concentration
Effects on fish			
Onchorhynchus mykiss	Bti	96h	LC_{50}(96h): >370 mg/L
Lepomis macrochirus	Bti	96h	LC_{50}(96h): >600 mg/L
Onchorhynchus mykiss	VectoBac technical	32 d	no adverse effects (water: 1.1x10^{10} CFU/L; 3.7x10^5 ITU/L); diet: 1.72x10^{10} CFU/g ; 5.7x10^5 ITU/g); fish growth in the VectoBac treatment significantly lower than in control, due to high turbidity and suspended solids
Lepomis macrochirus	VectoBac technical	30 d	no adverse effects (water: 1.2x10^{10} CFU/L ; 4x10^5 ITU/L; diet: 1.31x10^{10} CFU/g; 4.4x10^5 ITU/g)
Cyprinodon variegatus	VectoBac technical	30 d	no adverse effects (water: 1.3x10^{10} CFU/L; 4.3x10^5 ITU/L; diet: 2.1x10^{10} CFU/g; 7x10^5 ITU/g)
Effects on freshwater invertebrates			
Daphnia magna	VectoBac Technical	10 d	NOEC >50 mg /L (1x10^{10} CFU/L)
Daphnia magna	VectoBac Technical	21 d	LOEC: 5 mg/L (1x10^9 CFU/L; 3.3x10^4 ITU/L); NOEC: 0.5 mg/L (1x10^8 CFU/L; 3.3x10^3 ITU/L)
Grass shrimp (*Palaemonetes vulgaris)*	VectoBac Technical	31-d	NOEC > 2.0 x 10^{10} CFU/(6.6 x 10^5 ITU/)
Mayfly nymphs (*Hexagenia* sp)	VectoBac Technical	18-d	NOEC > 2.0 x 10^{10} CFU/L
Amphiascus minutus	VectoBac Technical	10-d	NOEC > 1x10^{10} CFU/L (3.3x10^5 ITU/L)
Effects on algal growth			
Euglena ssp.; Chlamydomonas sp.; Oedogonium sp.; mixed algal cultures; Oscillatoria sp. (cyanobacterium)	Toxins from Bti	n.a.	no adverse effects

Effects on aquatic plants			
No data presented			

Ref-MS information to the reader:	For all species in the Table, the endpoint stated in ITU is considered not relevant. These species are not sensitive to the crystal proteins and the numbers given in the table is a direct translation from the endpoint given as CFU.

2.5.2 Terrestrial compartment

Bti AM65-52 is not considered to be acutely toxic to earthworms. A 30-day earthworm acute gave an LC_{50} value of >1000 mg/kg dry weight soil. Exposure was via soil and treated food. Under the conditions of the study, VectoBac technical powder (*Bti*) was neither toxic nor pathogenic to the earthworm *Eisenia fetida.*

The end-points for the terrestrial compartment are summarised in Table IIB 5.2-1

Table 2.5.2-1 – Bti Strain AM6552/VectoBac G - endpoints for the terrestrial compartment

Test organism	Test substance	Duration	Effective Concentration
Effects on birds			
Mallard duck	VectoBac Technical	5-days	LD_{50}>3077 mg/kg bw day (6.2 x 10^{11} CFU/kg bw day; 2.03 x 10^7 ITU/kg bw day); no pathogenicity, nor infectivity observed
Northern bobwhite	VectoBac Technical	5-days	LD_{50}>3077 mg/kg bw day (6.2 x 10^{11} CFU/kg bw day; 2.03 x 10^7 ITU/kg bw day); no pathogenicity, nor infectivity observed
Effects on earthworms			
Eisenia fetida	VectoBac Technical	30-days	30-day LC_{50} > 1000 mg/kg $_{soil}$ (4.8x10^{10} CFU/kg ~~soil~~; 8x10^6 ITU/ kg $_{soil}$) no toxic nor pathogenic effect observed
Effects on soil non-target micro-organisms			
Bacillus megaterium, B. subtilis, B. cereus, Staphylococcus faecalis S. aureus	Bti toxins	--	No effects observed
Effects on honey bee			
Apis mellifera (adult workers)	VectoBac Technical	14-days	No effect was observed in tests on oral toxicity test carried out at a maximum dose of 124.1 µg/bee/day (6x10^6 CFU/bee/day; 3.9x10^4 ITU/bee/day)
Effects on terrestrial plants			
No test carried out	--	--	--

Field application of two commercial formulations of *Btk* at application rates of 6000 mg/m^2 and 30 g/m^2 respectively, had no effect on earthworm density nine weeks after application. *B. thuringiensis* subsp. *kurstaki* and *Bti* are both ubiquitous soil micro-organisms and earthworms will be continuously exposed to low levels of these bacteria. The lack of adverse effects in earthworms following treatment with *B. thuringiensis* subsp. *kurstaki* at high levels is considered to be indicative of the general safety of *B.thuringiensis* species to earthworms and there is no expectation that adverse effects would be observed following a similar treatment with *Bti*.

The results of the two short-term dietary studies with 'VectoBac' technical material indicate that 'VectoBac' technical material is non-toxic to birds.

A 14-day oral toxicity study was conducted to determine the effects of 'VectoBac' technical material (*Bti* AM65-52) on adult worker honey bees. The result of the study showed that 'VectoBac' was not a stomach poison to adult worker honey bees (*Apis mellifera* L.) when fed at doses up to 10x the field rate (2400 g /acre; 5931 g/ha). On the basis of these results 'VectoBac' can be classified as essentially non-toxic to honey bees.

On the basis of this results 'VectoBac' G can be classified as essentially not-toxic to honey bees.

No studies have been performed with terrestrial plants. Plants are not considered to be at risk 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.

2.5.3 Atmosphere

The results of numerous surveys indicate that *Bti* is a naturally occurring microbe present at low levels in the environment. The vegetative cells and insecticidal toxins of *Bti* are readily 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 for extended time periods and consequently the micro-organism will not undergo long-range atmospheric transportation.

There are no formulation components or properties of the formulation that are considered to affect the behaviour of the active micro-organism, *Bti* (Strain AM65-52), in the atmosphere and an exposure assessment in air for 'VectoBac' G has not been conducted.

2.5.4 Non compartment effects relevant to the food chain (primary and 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 the 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.

Ref-MS information to the reader:	For the entire Section 2.5, it is acceptable to use the non-formulated strains in all risk assessments as the formulations as such are not likely to affect the toxicity. Endpoints are correctly taken from the CAR. No risks are observed in the atmosphere and to secondary effects in the food chain. The Christensen, 1990 report has been evaluated in the CAR with ref number IIIA, 8.2.2/03.

2.6 HAZARD IDENTIFICATION FOR BIOLOGICAL PROPERTIES

'VectoBac' G does not possess any physico-chemical properties that indicate a particular physical hazard during storage, transport or use and therefore it is not classified under Directive 67/548/EEC on the basis of its physico-chemical properties.

The active micro-organism in 'VectoBac' G, *Bacillus thuringiensis* subsp. *israelensis*, Serotype H-14, Strain AM65-52, poses no quantifiable risk to human health. The use of 'VectoBac' G is therefore unlikely to cause human disease and the product can be classified as a Group 1 biological agent according to Article 2 of Directive 2000/54/EC. The use of the biohazard symbol on the 'VectoBac' G product label is not required.

The micro-organism *Bti* Strain AM65-52 is regarded as a potential skin and respiratory sensitiser according to the conclusions presented in the Annex I Assessment Report. As a consequence product labels for 'VectoBac' G require the following phrase:

Contain Bacillus thuringiensis, micro-organisms may have a potential to provoke sensitising reactions

S24/25: Avoid contact with skin and eyes

S36/37: Wear appropriate protective clothing and gloves.

Ref-MS information to the reader:	The text in Section 2.1.4 concerning Classification and Labelling is in agreement with the Annex 1 Assessment Report (CAR) and KemI do not oppose to the proposed precautionary statements in principle. However, in section 2.6 the applicant suggests different phrases; S24/25 and S36/37, and in the submitted application form the phrases P262 and P280 from the CLP-regulation are suggested. After consulting the applicant we have agreed on the following precautionary statements from the CLP-regulation: P261: Avoid breathing dust. P280: Wear protective gloves/protective clothing P302 + P352: IF ON SKIN: Wash with plenty of soap and water. P363: Wash contaminated clothing before reuse. For more information regarding the biological properties see section 2.1.

2.7 RISK CHARACTERISATION FOR HUMAN HEALTH

Data presented in this product dossier have been derived from two similar versions of the granule product; VectoBac GR and VectoBac G. The two products are very similar in their construction and composition. They both contain the same amount of active substance and carrier absorbed onto a natural granular support. The granular support is a carrier and is basically inert. It is therefore reasonable to consider that the toxicological and ecotoxicological characteristics of the two products will be similar. Therefore, throughout this dossier, wherever 'VectoBac' G is stated, reference to 'VectoBac' GR is also inferred.

2.7.1 General aspects

Human health effects assessment

The uses and product supported in this dossier are the same uses that were evaluated and concluded in the Annex I Assessment Report for *Bti* Strain AM65-52 (February 2010); the product 'VectoBac' G is also very similar to the representative product 'VectoBac' WG. The summary of risks posed to human health from the active microorganisms, presented in the Assessment Report, stated the following:

There is no evidence that *Bti* Strain AM65-52 could lead to infections in humans, so it has to be considered safe with the precautionary exception to prevent the exposure of immune suppressed subjects which must be considered at risk.

Bti Strain AM65-52 technical powder could induce sensitisation in animal models, although current animal models are not considered appropriate for microbial products. Human data and epidemiological records from spray campaigns have not presented sensitization evidence. While the risk of sensitization and / or allergenicity in humans cannot be excluded, as for all microbes currently '*Bti* Strain AM65-52 should be considered as a potential human sensitizer. Therefore, the product 'VectoBac' G should be labelled with safety phrases such as: avoid contact with skin, wear gloves when handling the product and do not breathe dust. It should not be labelled with the risk phrase Xi on the basis that the guideline studies do not show 'VectoBac' G to be a sensitiser.

The conclusions regarding the health effects of *Bti* Strain AM65-52 are relevant and appropriate for the authorisation of 'VectoBac' G and further information should not be necessary.

2.7.2 Professional users

2.7.2.1 Active substance

2.7.2.2 Critical endpoint(s)

Since the testing of *Bti* Strain AM65-52 is largely limited to acute exposure, based on the short term activity of endotoxins and the non-pathogenic nature of the bacteria, there are no data from which to derive conventional values for ADI or AOEL. For the same reasons no maximum allowable concentration (MAC) in drinking water has been calculated. *Bti* Strain AM65-52 is not used on finished treated drinking water.

2.7.2.3 Relevant exposure paths

Bacillus thuringiensis products for Valent BioSciences Corporation are made by Abbott Laboratories. Potential inhalation, oral or dermal exposures during manufacture, packing, cleaning or maintenance are subject to Abbott Laboratories engineering controls, administrative procedures designed to prevent exposure and the wearing of protective equipment in accordance with industrial health and safety legislation. The potential for exposure to *Bti* Strain AM65-52 is therefore negligible.

2.7.2.4 Risk characterisation for production / formulation of a.s.

The risk to professional workers is negligible. No adverse reactions in individuals as a result of contact with *Bti* Strain AM65-52 during its development, manufacture, preparation or field application have been documented or reported. There have been no medical surveillance abnormalities or reports to the Occupational Health Services at Abbott Laboratories from Abbott employees to date regarding health related or other adverse reactions.

2.7.3 Biocidal product

2.7.3.1 Critical end point(s)

The Annex I Assessment Report for *Bti* Strain AM65-52 (February 2010) concluded that *Bti* Strain AM65-52 poses no quantifiable risk to human health in respect of its use as a microbial insecticide. There are no co-formulants or characteristics of the product 'VectoBac' G that would alter this conclusion and the setting of critical endpoints for the biocidal product 'VectoBac' G is therefore not considered relevant.

2.7.3.2 Relevant exposure paths

'VectoBac' G is used for the control of mosquito and black fly larvae in water habitats such as ditches, pools, pastures, water retention areas, salt marshes and standing water in crops fields (including rice and orchards) and filter fly midges in sewage treatment plants. The potential for professional workers to be exposed to 'VectoBac' G during use is summarised below:

Inhalation exposure

'VectoBac' G is a granular (G) formulation. Professional users could be exposed by inhalation during mixing/loading and during application. However, the formulation is non-dusty which will reduce the potential for inhalation exposure during mixing/loading or application. Professional users are required to wear a dust/mist filtering respirator to reduce inhalation exposure during mixing/loading and during application. Only professional users wearing protective equipment are permitted in areas being treated.

Oral exposure

'VectoBac' G is not likely to reach the mouth of professional users (the required use of mist filtering respiratory masks effectively precludes oral exposure).

Dermal exposure

'VectoBac' G is a granular formulation. Professional users could be exposed dermally during mixing/loading and during application. However, the formulation is a granule which will reduce the potential for dermal exposure of the hands during mixing/loading as the particles will not adhere to gloved hands. Users are required to wear long-sleeved shirt, long trousers, shoes and socks, and water-proof gloves to reduce dermal exposure during mixing/loading and application. Only professional users wearing protective equipment are permitted in areas being treated.

2.7.3.3 Risk characterisation for the biocidal product

The potential for professional workers to be exposed to *Bti* Strain AM65-52 is small due to the physical nature of the product and the use of personal protective equipment during mixing/loading and application. *Bti* Strain AM65-52 poses no quantifiable risk to human health and therefore the likelihood of adverse health effects occurring in humans through inadvertent inhalation, dermal or oral exposure will be negligible.

2.7.4 Overall assessment of the risk to professionals for the use of the active substance in the biocidal product

Bti Strain AM65-52 poses no quantifiable risk to human health and the risks to professional workers through either manufacture or use of the active micro-organism or formulated product VectoBac' G are negligible.

2.7.5 Non-professional users

There are no non-professional uses proposed for Vectobac G. Consumer and bystander exposure is negligible due to a combination of no direct contact through use and by exclusion of non-professionals from areas being treated.

2.7.6 Indirect exposure as a result of use

2.7.6.1 Active substance

2.7.6.2 Critical endpoint(s)

Since the testing of *Bti* Strain AM65-52 is largely limited to acute exposure, based on the short term activity of endotoxins and the non-pathogenic nature of the bacteria, there are no data from which to derive conventional values for ADI or AOEL. For the same reasons no maximum allowable concentration (MAC) in drinking water has been calculated. *Bti* Strain AM65-52 is not used on finished treated drinking water.

2.7.6.2.1 Relevant exposure paths

Bti Strain AM65-52 is made by Abbott Laboratories under strict engineering and procedural control and the possibility of indirect exposure to *Bti* (Strain AM65-52) during manufacture is negligible.

2.7.6.3 Risk characterisation for production / formulation of a.s.

Bti (Strain AM65-52) poses no quantifiable risk to human health and the possibility of indirect exposure during manufacture is negligible due to the controls in place in the manufacturing plant.

2.7.6.4 Biocidal product

2.7.6.4.1 Critical end point(s)

The Annex I Assessment Report for *Bti* Strain AM65-52 (February 2010) concluded that *Bti* Strain AM65-52 poses no quantifiable risk to human health in respect of its use as a microbial insecticide. There are no co-formulants or characteristics of the product 'VectoBac' G that would alter this conclusion and the setting of critical endpoints for the biocidal product 'VectoBac' G is therefore not considered relevant.

2.7.6.4.2 Relevant exposure paths

Inhalation exposure

Non-users are not expected to be close during application, non-professionals are excluded from treated areas * The risk of inhalation exposure of non-users to drift during application or to residues after application via the environment is considered to be negligible. The granules have been developed to have enough weight to reach mosquito breeding sources.

Dermal exposure

Non-users are not expected to be close during application. The risk of dermal exposure of non-users to spray drift during application or to residues after application via the environment is considered to be negligible.

Oral exposure

Bti (Strain AM65-52) is not used directly on food or feed commodities and is not used on water bodies which are treated drinking water. The risk of oral exposure to residues during or after application is therefore considered to be negligible. Treatment of standing water in which crops are grown (rice) is withdrawn at least one month prior to harvest.

2.7.6.4.3 Risk characterisation

Non-users are not expected to be close during application of 'VectoBac' G and will not be exposed to commodities or drinking water containing residues of the active micro-organism *Bti* Strain AM65-52. The possibility of indirect exposure to *Bti* Strain AM65-52 is therefore extremely small. *Bti* Strain AM65-52 poses no quantifiable risk to human health and therefore the likelihood of adverse effects occurring in humans through any indirect exposure will be negligible.

2.7.6.5 Overall assessment of the risk to non-users for the use of the active substance in biocidal product

Bti Strain AM65-52 poses no quantifiable risk to human health and the risks to non-users through indirect exposure to the product 'VectoBac' G are negligible.

2.7.7 Combined exposure

Bti Strain AM65-52 poses no quantifiable risk to human health and the risks to professional workers through either manufacture or use of the active micro-organism or the formulated product 'VectoBac' G are negligible. *Bti* Strain AM65-52 poses no quantifiable risk to human health and the risks to non-professional users through

use of the formulated product 'VectoBac' G are negligible. *Bti* Strain AM65-52 poses no quantifiable risk to human health and the risks to non-users through indirect exposure to the product 'VectoBac' G are negligible.

Ref-MS information to the reader:	The conclusion by KemI is that the risks to human health are considered acceptable if all of the precautions indicated above and listed in Section 9 are followed. The period of one month between last treatment and harvest of crops grown in standing water is considered sufficient. It is also essential that all precautions stated in the confidential information, such as routine screening for contaminants are followed. In correspondence with the applicant concerning the safety of the product especially when applied to crops we got the following answer ""The rationale for the one month period prior to harvest in which treatment of crops growing in standing water (e.g. rice) should not be treated should not be considered strictly speaking as a pre-harvest interval. This timing is based on the time in which the water surround the crops is allowed to dry out prior to harvest. Since no water is present mosquitoes no longer have a breeding site. It should also be remembered that the granules are heavy enough to go through vegetation to reach the water where the mosquito larvae are present. This is not a spray of a crop. Please see attached study of microbial numbers with VectoBac G, and the reductions seen and the rapid DT50. As in the top water, B.t.i. spores in bottom water were found in high numbers of 7.75 x 10^3 and 10.0 x 10^3 spores/ml at three hours after treatment with VectoBac G at the rates of 5.3 and 10.6 Ib/ac, respectively, then gradually declining to 6.30 x 10^2 and 1.36 x 10^3 spores/ml, respectively, on day 7 post treatment." KemI considers this information helpful for the overall risk assessment. However, there is a hypothetical concern, not assessed by the applicant, when VectoBac treated irrigation water is used on growing crops. Watering crops with recently treated irrigation water is considered not different to adding VectoBac directly to water and food used for human consumption and therefore not allowed according to the proposed use. The possibly risk scenario is then when irrigation water is applied on soil and hence may be present at soil vegetables. In a very conservative approach, the accumulated level of Bti in soil would be equal to the level reached when VectoBac is applied directly to soil. After 8 applications the concentration reaches 1.4*10^8 CFU/kg. Normally, *Bti* is not colonising the plant but assuming the final concentration, attached to, or within, the plant is 10% compared to the surrounding soil. Then the final concentration is 1.4*10^4 CFU/g. This concentration is not considered critical according to the human health risk assessment performed presented in the CAR. Also, background levels of related bacteria ar far greater. Hence, the overall conclusion is that there is no specific risk when applying VectoBac to irrigation water. * This sentence is incorrect but we interpret the text as non-professionals are not present when the product is applied.

2.8 RISK CHARACTERISATION FOR THE ENVIRONMENT

The uses of 'VectoBac' G are largely the same as those that were evaluated in the Annex I Assessment Report for *Bti* Strain AM65-52 (February 2010). However, as the concentration of the active ingredient and the application rate differ, PECs have been derived separately for 'VectoBac' G (see Doc. IIB). In terms of the effects assessment it is considered that the formulation of 'VectoBac' G does not affect the behavior of the microorganism and therefore the data collected for *Bti* Strain AM65-52 is directly applicable. Hence, the risk characterisation presented below is based PECs/EEDs derived specifically for 'VectoBac' G and PNECs/PNEDs derived for the active ingredient.

Direct application to surface waters, STPs, and drift to soils was considered in this risk assessment to cover all potential uses of the product.

Ref-MS information to the reader:	KemI agrees that risk assessment on direct application to surface waters, STP (sewage treatment plants), and drift to soils cover all potential uses of the product.

2.8.1 Aquatic compartment (incl. sediment)

In the studies conducted, *Bti* AM65-52 is not considered to be acutely toxic to fish and there is no evidence of significant effects following long-term exposure. Similarly *Bti* AM65-52 is not considered to be acutely toxic to aquatic invertebrates; there is no evidence of significant effects following long-term exposure. However, the results of tests on aquatic organisms (fishes and Daphnia, in particular) carried out in the lab with high concentrations of product, could be affected by the high turbidity of water due to the product suspension. A study was conducted using toxins from *Bti* (25 – 130 kDa) which were purified from 3 -5 day old cultures. The tests were performed with *Euglena* spp, *Chlamydomonas* sp., *Oedogonium* sp and mixed algal cultures and a cyanobacterium (*Oscillatoria* sp). The conclusion of the tests was that the toxins were not inhibitory in dilution tests to pure and mixed cultures of algae or to the cyanobacterium. No studies have been performed with aquatic plants; a single study with algae was reported as showing no effect. However, plants and algae are not considered to be at risk as there is no mechanism for the ingestion of *Bti* AM65-52 and therefore no appropriate digestive enzymes exist to enable the release of the active protein δ-endotoxins.

The $PNEC/PNED_{sw}$ is derived in the Annex 1 CAR using the NOEC of 0.5 mg/L (1 $x10^8$ CFU/L) obtained in a Daphnia reproduction test. An assessment factor of 10 was applied to this to give a $PNEC/PNED_{sw}$ of 0.05 mg/L (1 $x10^7$ CFU/L).

The step 1 results for surface water following 1 and 8 applications of 'VectoBac' G for mosquito control are presented below in Table 2.8.1-1.

Table 2.8.1-1 - Aquatic compartment - EEDsw/PNECsw ratios for mosquito control uses (Step 1)

Test organism	NOEC (21 d, chronic)	AF	PNECsw [mg/L]	Step	EEDsw [CFU/L]	EEDsw/PNEDsw
Daphnia magna	0.5 mg/L (1x10^8 CFU/L)	10	0.05 (1x10^7 CFU/L)	1	1 application: 6.7 x10^6	**0.67**
					8 applications: 3.9 x10^7	**3.9**

As the EED/PNED ratio after 8 applications is greater than 1, it is considered that safe use was not demonstrated for surface waters in step 1. As such, refined step 2 calculations were conducted using a simple two-compartment model which considered degradation in the water phase and adsorption in the sediment phase. The resultant EEDs and EED/PNED ratios are presented in Table 2.8.1-2.

Table 2.8.1-2 - Aquatic compartment - EEDsw/PNECsw ratios for mosquito control uses (Step 2)

Test organism	NOEC (21 d, chronic)	AF	PNECsw [mg/L]	Step	EEDsw [CFU/L]	EEDsw/PNEDsw

Daphnia magna	0.5 mg/L ($1x10^8$ CFU/L)	10	0.05 ($1x10^7$ CFU/L)	2	1 application: $4.9 x10^5$	**0.049**
					8 applications: $2.9 x10^6$	**0.29**

Following step 2 calculations for the PEC/EED, the EED/PNED ratio was found to be less than 1 for 1 and 8 applications of 'VectoBac' G. Consequently, it is considered that there is no significant risk to surface waters.

Predicted EED_{sed} values are substantially lower than the corresponding EED_{sw} values for all scenarios. Based on this lower exposure and the specific mode of action to larvae of certain *dipteran* insect species negligible risks are expected in the sediment environment. As such, it is not considered necessary to conduct an assessment for the sediment compartment.

A Daphnia test for microbials is being developed as the nature of the current guidance for chemicals poses an unrealistic scenario.*

Ref-MS information to the reader:	KemI agrees with the conclusions that there is no risk to algae, fishes and aquatic plants. The risk to Daphnia in surface water is considered acceptable if the step 2 refinements can be accepted. Calculations relevant for the Risk Assessment for *Daphnia* can be found in Sections 2.3 and 2.8. KemI also agrees with the statement that no assessment for the sediment is necessary as the concentration is lower in sediments compared to surface water. However, sensitive dipteran species within the sediment could be affected, but as the concentration is rather low, the crystals rapidly degraded, and only effective against larvae, it is considered unlikely that any non-target insect population can be significantly reduced. Evidently, no significant long-term effect has been observed even on target larvae as treatment has to be repeated to control the population. To further address the potential concerns with *Daphnia*, the applicant has provided a few peer-reviewed publications (Vaishnav & Anderson, 1995; Duchet et al. 2008, Duchet et al. 2011) where the risk to *Daphnia* is claimed to be low. However, KemI´s opinion is that the results from these studies are not directly transferrable and there is a significant risk. After the above-mentioned step 2 refinement, the numbers of *Bti* even after multiple applications are reduced to acceptable levels. Nevertheless, with an interval of one week between applications, the spores in water are assumed to either be sedimented, germinated or consumed, i.e. the accumulation is likely not significant. Based on an overall weight of evidence approach conducted by KemI, the risk to *Daphnia* is considered acceptable. *The chronic low risk indicated in *Daphnia* is probably not being connected to the crystal proteins as no acute effect was observed. Consequently, the risk has to be calculated from CFU rather than ITU. Likely the toxicity effect is derived from secondary effects caused by the high concentration of formulation additives in the test medium, or by other weaknesses in the experimental procedure, as the test is not designed for microorganisms.

2.8.2 Sewage treatment plants (STP)

A study 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. No antibiotic activity of the ICPs from *Bti* against a variety of gram-positive bacteria was observed.

In conclusion, there is no expectation that the use of 'VectoBac' G will have an adverse effect on the microbial activity occurring in sewage treatment plants.

2.8.3 Atmosphere

The results of numerous surveys indicate that *Bti* is a naturally occurring microbe present at low levels in the environment. The vegetative cells and insecticidal toxins of *Bti* are readily degraded and although spores of *Bti* are more resistant they do not multiply substantially. Due to the relative instability of *Bti* in the environment, *Bti* (Strain AM65-52) substantial concentrations of the micro-organism will not be present in air for extended time periods and consequently the micro-organism will not undergo long-range atmospheric transportation.

2.8.4 Terrestrial compartment

Avian Risk Assessment

The results of the two short-term dietary studies with 'Vectobac' technical material indicate that 'Vectobac' technical material is non-toxic to birds (according to the US EPA toxicity categories for dietary studies). In addition there was no apparent pathogenicity after a 25 day observation period. The lack of likely effects on avian species is further suggested by the specificity of the mode of action of *Bti* AM65-52 which requires alkaline gut conditions of pH 9.0 – 10.5. The pH of avian *Bacillus thuringiensis* subsp. intestinal tracts is slightly acidic so even if ingestion of *Bti* AM65-52 occurs there will be no exposure to the active protein δ-endotoxins. In addition, the results of numerous surveys indicate that *Bti* is a soil microbe as well as an inhabitant of the phylloplane, therefore birds can be exposed to low levels of *Bti* through their normal diet.

Earthworm risk assessment

Bti AM65-52 is not considered to be acutely toxic to earthworms. A 30-day earthworm acute gave an LC50 value of >1000 mg/kg dry weight soil. Under the conditions of the study, Vectobac technical powder (*B. thuringiensis* subsp. *israelensis*) was neither toxic nor pathogenic to the earthworm *Eisenia fetida*. A study conducted with two commercial formulations of *B. thuringiensis* (Dipel and Bactospeine) at application rates of 6000 mg/m^2 and 30 g/m^2 respectively, concluded that neither product had any effect on earthworm density nine weeks after application. The lack of likely effects on earthworms is further confirmed by the specificity of the mode of action of *Bti* AM65- 52 which requires alkaline gut conditions of pH 9.0 – 10.5. The pH of earthworm intestinal tracts is neutral so even if ingestion of *Bti* AM65-52 occurs there will be no exposure to the active protein δ-endotoxins. In addition the results of numerous surveys indicate that *Bti* can be found in soil and therefore earthworms can be naturally exposed to low levels of *Bti* in their natural habitat.

Bees risk assessment

A 14-day oral toxicity study conducted with 'VectoBac' technical material on adult worker honey bees (*Apis mellifera* L.) showed that 'VectoBac' was not a stomach poison to adult worker honey bees at dosages ranging up to 2400 g/acre (5931 g/ha; 2.85 x 10^9 CFU/ha). On the basis of these results 'VectoBac' can be classified as essentially not-toxic to honey bees. The lack of likely effects on non-target species is further confirmed by the specificity of the mode of action of *Bti* AM65-52 which requires alkaline gut conditions of pH 9.0 – 10.5 (as detailed in the introduction). In a laboratory study where bees were fed *Bti* AM65-52 for 14 days at rates up to 2400 g/acre (10 times the recommended field application rate) with no adverse effects. In addition the results of numerous surveys indicate that *Bt*, possessing minimal growth requirements, is a fairly ubiquitous soil microbe as well as an inhabitant of the phylloplane, therefore bees can be exposed to low levels of *Bt*.

Terrestrial plants risk assessment

No studies have been performed with terrestrial plants. Plants are not considered to be at risk 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.

EED/PNED calculation for terrestrial compartment

'VectoBac'G is not applied directly to soil and the potential for indirect exposure is expected to be low. However, for the sake of completeness PEC/EED_{soil} and PNEC/$PNED_{soil}$ values were derived. The PNEC and PNED for terrestrial organisms were calculated in the Annex 1 CAR using the 30-day EC_{50} for earthworms of 1000 mg/kg soil. An assessment factor of 1000 was applied to give a PNEC of 1 mg/kg soil, which equates to a PNED of 1 $x10^7$ CFU/kg soil. Results of the terrestrial assessment are presented below in Table 2.8.4-1.

Table 2.8.4-1 - Terrestrial compartment – $EED_{soil}/PNEC_{soil}$ ratios for considering direct application of 'VectoBac' G to soil

Test organism	EC (mg/kg soil)	AF	$PNED_{soil}$ [CFU/kg]	EED_{soil} [CFU/kg]	$EED_{soil}/PNED_{soil}$
Eisenia fetida	1000 ($4.8x10^{10}$ CFU/kg soil)	1000	1 ($1x10^7$ CFU/kg)	1 application: 2.688×10^4	0.003
				8 applications: 1.875×10^5	0.019

As the $EED_{soil}/PNED_{soil}$ ratios are less than 1 for both 1 and 8 applications not further refinements are considered necessary.

The overall risk to the terrestrial compartment is considered to be negligible. It should be noted that this scenario represents a worst-case as 'VectoBac' G will not be applied directly to soil and densities of *Bti* in soil resulting from the use of 'VectoBac'G are not expected to exceed the levels of *Bti* which naturally occur in soils.

Ref-MS information to the reader:	KemI agrees with the calculations and for most organisms (Section 8.2) no unacceptable risk for birds and terrestrial plants can be identified. The risk to bees is acceptable but there is a concern connected to other non-target insects susceptible to *Bti*. Anyhow, as the risks are only assessed up to 8 annual applications, this is the maximal number can be authorised. The risk to earthworms is not correctly performed indicating an unacceptable risk after 8 applications. The calculated EED-values (page 25) are given in CFU/g but the correctly used PNED is in CFU/kg. The applicant has made new assumptions and calculated a novel EED_{soil} after one application to $2.8*10^6$ CFU/kg a number resulting also in an acceptable level after 8 applications. However, this refinement cannot be accepted as the soil volume/ha is considered to be 10 000 m^3, i.e. a thickness of one meter instead of 5 cm as originally stated. When KemI recalculated the EED-values using a correct soil density of 1.7 g/ cm^3 the initial EED value is $2.4*10^7$ CFU/kg resulting in a $EED_{soil}/PNED_{soil}$ of 0.5. After 8 applications the EED value is $1.4*10^8$ CFU/kg resulting in a $EED_{soil}/PNED_{soil}$ of 2.9, i.e. a non-acceptable risk. They applicant supplied an argumentation and correctly indicating that there is a natural level of *Bti* present in soil and that the active protein δ-endotoxins are not toxic to earthworms. However, the latter argument is not valid as a risk connected to CFU levels is indicated. Of greater importance, also stated in the correspondence is that VectoBac should only be applied to water bodies, not soil and as granules the drift is

	considered insignificant. The potential problem is when applied aerially. VectoBac may be applied on flooded areas and during withdrawal of water Bti may be accumulated to soil. The precision when applied aerially can also be considered lower than when applied from ground. There is no standard scenario for that particular kind of drift why a plausible approximation has to be performed with a precautionary approach. After evaluating the argumentation, the risk to earthworms is considered acceptable under normal conditions but when applied aerially accidental spillage is likelier indicating risk mitigation measures or a restricted number of allowed applications. When calculating the EED with the restrictions of 4 applications with 14 days interval instead of 7, the resulting EED value is $8.2*10^7$ CFU/kg resulting in a $EED_{soil}/PNED_{soil}$ of 1.7 also not acceptable. However, with the plausible assumption that not more than 50% of the product is applied to soil instead of water, the risk is considered acceptable.

2.9 MEASURES TO PROTECT MAN, ANIMALS AND THE ENVIRONMENT

2.9.1 Recommended methods and precautions concerning handling, use, storage, transport or fire	
2.9.1.1 Methods and precautions concerning placing on the market	User should comply with the user instructions. Users should only purchase sufficient quantities to use in one season and avoid storage for extended periods.
2.9.1.2 Methods and precautions concerning handling and use	Store under cool, dry and well-ventilated conditions. Keep away from food, drink and animal feed stuffs. Keep away from heat.
2.9.1.3 Methods and precautions concerning storage	Store under cool, dry and well-ventilated conditions. Keep away from food, drink and animal feed stuffs. Keep away from heat.
2.9.1.4 Methods and precautions concerning transport	There are no restrictions for *Bti* Strain AM65-52 or 'VectoBac' G concerning transport by land, sea or air.
2.9.1.5 Methods and precautions concerning fire	In case of fire use extinguishing media appropriate to surrounding conditions: dry chemical powder, carbon dioxide, foam, sand, or water are all suitable.
2.9.2 Specific treatment in case of an accident, e.g. first-aid measures, antidotes, medical treatment if available; emergency measures to protect the environment	
2.9.2.1 Specific treatment in case of an accident, e.g. first-aid measures, antidotes, medical treatment if available	Based on the recorded medical examinations at production facilities, the clinical observations or lack of effects in animal studies, the absence of known poisoning symptoms after several decades of *Bti* use as a microbial pesticide and the inability of the parasporal body to produce endotoxins under the intestinal conditions of warm-blooded species, *Bti* Strain AM65-52 is considered non-toxic and first aid measures and a specific therapeutic regimen can not be recommended. EYES: Remove from source of exposure. Flush with copious amounts of water. If irritation persists or signs of toxicity occur, seek medical attention. Provide symptomatic/supportive care as necessary. SKIN: Remove from source of exposure. Flush with copious amounts of water. If irritation persists or signs of toxicity occur, seek medical attention. Provide symptomatic/supportive care as necessary. INGESTION: Remove from source of exposure. If signs of toxicity occur, seek medical attention. Provide symptomatic/supportive care as necessary. INHALATION: Remove from source of exposure. If signs of toxicity occur,

2.9.1 Recommended methods and precautions concerning handling, use, storage, transport or fire	
	seek medical attention. Provide symptomatic/supportive care as necessary. TREATMENT: Supportive therapy, antibiotics may be used.
2.9.2.2 Emergency measures to protect the environment	*Bti* (Strain AM65-52) is harmless to non-target species and specific measures to protect the environment are not necessary.
2.9.3 Procedures, if any, for cleaning application equipment	Application equipment should be cleaned using normal cleaning procedures.
2.9.4 Identity of relevant combustion products in cases of fire	*Bti* Strain AM65-52 is not flammable or oxidising. None of the components in 'VectoBac' G contain halogens. In the event of a fire 'VectoBac' G is likely to produce normal products of combustion i.e. oxides of carbon. It is not anticipated that significantly toxic, irritating or corrosive products will be formed.
2.9.5 Procedures for waste management of the biocidal product and its packaging and where relevant, treated waste material for industry, professional users and the general public (non-professional users), e.g. possibility of reuse or recycling, neutralisation, conditions for controlled discharge, and incineration	*Bti* Strain AM65-52 and any associated contaminated packaging should be disposed of by incineration, or in accordance with governmental or local authority regulations. These are standard procedures and no further detailed instructions are required. If further advice is required contact the manufacturer.
2.9.6 Possibility of destruction or decontamination following release onto:	
2.9.6.1 Air	*Bti* Strain AM65-52 is harmless to non-target species and humans and therefore no special requirements are needed to render the micro-organism harmless.
2.9.6.2 Water, including drinking water	*Bti* Strain AM65-52 is harmless to non-target species and humans and therefore no special requirements are needed to render the micro-organism harmless.
2.9.6.3 Soil	*Bti* Strain AM65-52 is harmless to non-target species and humans and therefore no special requirements are needed to render the micro-organism harmless.
2.9.7 Observations on undesirable or unintended side-effects, e.g. on beneficial and other non-target organisms	*Bti* Strain AM65-52 is harmless to non-target species and humans and no undesirable or unintended side effects are anticipated.
2.9.8 Specify any repellents or poison control measures included in the preparation that are present to prevent action against non-target organisms	Not applicable. *Bti* Strain AM65-52 does not contain any repellents or poison control measures in the preparation.

Ref-MS information to the reader:	KemI agrees with the recommended methods and precautions in the Table above. However, the term "harmless" (in points **2.9.2.2, 2.9.6 and 2.9.7)** is considered inappropriate. It should be read as "with the exception to other nematoceran species, there is no evidence of harmful effects".

3 PROPOSAL FOR DECISION

Here the decision for granting an authorisation or not should be presented and in any case justified. Also, if the authorisation is limited/restricted in some way the restrictions should be described and justified under this heading.

The "specific provisions" and "elements to be taken into account by Member States when authorising products" from the assessment report(s) of the active substance(s) shall be duly taken into consideration. New data/information rendering these provisions and recommendations obsolete shall be explicitly referenced

3.1 BACKGROUND TO THE DECISION

3.1.1 From the Assessment Report

The active substance, *Bacillus thuringiensis* subsp. *israelensis* Serotype H-14 Strain AM65-52, has previously (2011-09-20) been included as an active substance in Annex I to directive 98/8/EC. Italy was the Rapporteur Member State. In the Assessment Report, the following elements should be taken into account by MSs when authorising products:

- Bti AM65-52 may cause a sensitisation reaction.

- The following uses have not been assessed: application to clean purified drinking water or water intended for direct human consumption; intentional spray of food crops, processed foods or surfaces likely to be used to store, process or present food; application for air sprays by planes, helicopters or others flying vehicles; and application by irrigation systems where overhead sprinklers are used.

- If direct application around food crops is made, a time interval between the last treatment and the re-entry of workers should be considered.

- When granting product authorisation, Member States will evaluate the possibility to assess the effects arising from long term and large scale use of the product on natural biological diversity, and eventually take appropriate measures to mitigate the identified risks.

- Label of products should indicate that the product should not be used by subjects affected by immunodeficiency, primary or secondary or in treatment with immunosuppressive agents, which can significantly reduce the effectiveness of the immune system response, unless it is demonstrated that such statement is not necessary.

- In case of application for amateur products, Member States will need to take account of the type of product and its use patterns, as well as its potential to cause skin sensitisation.

Requirement for further information:

It is considered that the evaluation has shown that sufficient data have been provided to verify the outcome and conclusions, and permit the proposal for the inclusion of *Bacillus thuringiensis* subsp. *israelensis* – Strain AM65-52 in Annex I to Directive 98/8/EC. However, when a suitable test protocol is available, a new study on *Daphnia* should be conducted since the reason for the effects seen in the present test must be elucidated.

3.1.2 Previous use and authorisation in Sweden

In Sweden VectoBac G has been used with temporary approvals since 2002. The current authorisation (2010-06-24, Dnr F-3422-227-10) is valid until 2014-12-31. Noteworthy, in Sweden, permission to apply VectoBac G aerially and over areas covered by the Natura 2000 network of protected areas or nature reserves is granted by the Swedish Environmental Protection Agency on yearly basis.

3.1.3 Risk assessment for aerial application of VectoBac

In the material submitted by the applicant, it is evident that the performed risk assessment for is primarily handling the risks connected to ground application. It is the opinion of KemI that, especially when assessing the environmental risk there are fundamental differences that need to be assessed when VectoBac is applied aerially. There is also an increased concern for individuals entering treated areas. Other potential risks of using VectoBac, for example, health risks to the user, are considered not dependent on how the product is spread.

When applied aerially, KemI has identified three fundamental differences, relevant to the environmental risk assessment not assessed by the applicant and previously in this document discussed briefly in the RMS commenting boxes:

1. The risk assessment to non-target species are in most respect conservative and are relevant also when applied aerially. However, the weight of evidence approach, used to determine the risk for earthworms is not considered acceptable when applied aerially.

2. It is not possible, with accuracy, to ascertain that only target Nematoceran species are present in the treated areas.

3. Considering the fact that, in most, or all, scenarios, the treated area is larger and inaccessible, it is harder to monitor the consequences of treatment, in terms of efficacy and consequences for non-target species. A plausible risk is that *Bti*-sensitive non-target insect populations are affected indirectly affecting the whole ecosystem

For all three concerns raised, there is no standard methodology to assess the risks. Standard risk mitigation actions, i.e. buffer zones are not considered feasible. When applied aerially greater areas are affected and the application will, in practice, not be restricted to water bodies, especially when applied on flooded areas as the water might have withdrawn at the time of application, but the larva are hatching in remaining puddles. Regarding the risk to earthworms, KemI refers to the commenting box at the end of section 2.8.4 where we propose that an acceptable usage can be achieved by reducing the frequency and number of applications. With this approach the risk is considered acceptable. However, there are strong arguments why the risk to earthworms should be considered low under all given circumstances. KemI agrees, at least partly, but there are other, more relevant arguments to reduce the frequency and numbers of annuals applications when considering the concerns above listed as 2 and 3.

Noteworthy, is that at no location treatment with *Bti* has ever even reduce the number of target mosquitos close to extinctions why KemI consider treatments over larger areas acceptable. However, although, in our opinion, not adequately supported by scientific reports, both the numbers of target and non-target Nematoceran may have indirect effect on non-related animal populations, e.g. fishes and birds. An additional period between the applications increases the likelihood of non-target sensitive insect populations to recover. Also, with just a one-week interval between the applications it is not possible to determine whether or not the previous application had any effect on the target mosquito population, why this restriction rather forces correct usage. A similar argumentation can be used if restricting the total number of annual applications. With this restriction, the applications will only occurs when the mosquito problems is at the most, allowing other, less dominant Nematoceran species to propagate. It must also be emphasised that, in several member states there the use of biocides in areas of great value for biodiversity, i.e. Natura 2000, and/or when applied aerially specific permits are

required when using biocidal products. Thus, there is room for stricter regulations depending on national opinions and regulations.

Considering increased health risks when applied aerially it is, dependent on the shape of the product and the relatively harmless properties of *Bti*, the additional risk is considered negligible unless individuals are directly exposed. Experienced pilots and calibrated equipment is necessary as well as proper information that keeps people away from the areas at time of application. The experience from the previous use in Sweden is that the compulsory information to bystanders, i.e. proper advertisement etc. has been sufficient to avoid exposure to non-protected individuals.

3.1.4 Conclusions

The risk assessment of VectoBac G and VectoBac GR has only been carried out on the intended use, i.e. to control of mosquito larvae in water habitats. Suggested concern for amateur products, application to drinking water, food etc. have therefore not been assessed. Individuals with a compromised immune system should not handle VectoBac but this may not require any specific action. We agree that the test to *Daphnia* is not suitable for microorganisms and the endpoint given is considered to be a conservative estimate and with an overall weight of evidence approach including the step 2 refinement we conclude that the risk to *Daphnia* is acceptable. The risk assessment is based on calculations of up to 8 annual applications with one-week intervals and this is therefore considered as the application plan proposed by the applicant. An increase in application number and frequency rather indicates misuse of the product than an actual need to control mosquito populations. Consequently, it is essential that the user is trained and skilled in identifying target species and larval stages. The granular VectoBac formulations are intended to be applied using conventional ground or aerial application equipment. With the proposed use, the risk to human health is considered low.

There are some concerns related to environmental risks. However, these concerns relate to secondary large-scale effects on biological diversity, mainly valid when VectoBac is applied to large areas. Although new studies would be beneficial, it is worth mentioning that *Bti* has been a target for numerous peer-reviewed studies investigating resistance development, effects to the ecosystem, human and animal toxicity. From these studies no immediate or mid-term threat when using *Bti* to reduce mosquito populations has been identified, and proposed long-term threats are based on hypothetical argumentation. These concerns are related to non-target insects also sensitive to *Bti*. The direct risk to all other species is considered negligible and there is no evidence of indirect consequences to the ecosystem. However, application of VectoBac should only be considered when there is, or going to be, a substantial mosquito problem, and there are good arguments to properly document all uses to facilitate research in and monitoring biodiversity. KemI is of the opinion that it is important to give local authorities an opportunity to take part in the planned activities regarding when and how the product is to be used in *natural* water habitats and, if authorised to do so, contribute with a local risk-benefit assessment case by case. Notably, when applying VectoBac to ecosystems of great value for biodiversity, i.e. Natura 2000, specific permission should be required. To summarise, the studies submitted by the applicant are considered sufficient to evaluate VectoBac in terms of safety and efficacy.

For the additional risks identified when VectoBac is applied aerially, KemI proposes some additional restrictions: Aerial application should only be allowed on larger areas where ground application is not feasible. Also, to assess the precautionary principle, the annual number of allowed aerial application will be restricted to four and an interval of at least 14 days between the applications. The information requirement should be equal to the requirements set for the previous Swedish approval, i.e. proper information to the public prior to aerial application. Moreover, at least in Sweden, special permission is required when distributing VectoBac aerially.

3.2 PROPOSAL FOR DECISION

On basis of the Assessment Report, the Product Report in combination with attached studies and the experiences of previous use in Sweden and worldwide, the opinion of KemI is to authorise the use of VectoBac G and VectoBac GR to be used as biocide products, with restrictions in line with the following:

- Recommended safety precautions should be followed.
- The protocols for routine screening for contaminants, products integrity etc. indicated in the confidential information should strictly be followed.
- The dosage interval of 2.5 -15 kg/ha is acceptable, but a time span of at least 1 week between applications is considered necessary to prevent misuse and reduce potential harmful effects on non-target organisms. For ground application not more than 8 annual applications are granted. For aerial application not more than 4 annual applications are granted. A period of at least 2 weeks between applications should be considered.
- Control of mosquitos with VectoBac G and VectoBac GR should only be performed when the target species eggs are hatched into larvae, during the 1st to the early 4th larval instar.
- VectoBac G and VectoBac GR should only be handled by professional and trained personnel that are able to distinguish between different larval germination stages. Wrongly used, e.g. applied outside the larval stage, the product is non-effective against target mosquitos but may theoretically reduce other insect populations. Therefore we suggest a condition in line with the following: The person responsible for the control shall ensure that the personnel involved in the control process have knowledge of 1. mosquitoes and their existence, both as larvae and adults; 2. handling of relevant application equipment; 3. how the prescribed dosage is achieved.
- VectoBac G and VectoBac GR should not be applied to food and water directly intended for human consumption.
- Individuals with a suppressed immune defence should not be in contact to VectoBac G and VectoBac GR.
- For applications on crops grown in standing water, the period between the last application and harvest must be at least one month.
- During all phases with potential exposures (including mixing/loading, application and post application phase such as cleaning of spraying equipment): wear sufficient dust filtering mask (with P3 filter or equivalent) or respirator, water-proof gloves (according to EN 374/2), protective clothing and goggles; or protecting technical measures (such as e.g. closed tractor cabs) may substitute PPE, if they provide the same degree of protection. Only professional users wearing protective equipment are permitted in areas under treatment.
- Application of VectoBac G and VectoBac GR should only be considered when there is, or going to be, a substantial mosquito problem. 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 G and VectoBac GR in a natural water habitat.
- To avoid exposure to bystanders, for at least 48 hours warning signs shall be placed on all entrances to treated area informing about the applied products.
- 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.

- When applying VectoBac G and VectoBac GR to ecosystems of great value for biodiversity, i.e. Natura 2000 or nature reserve, specific permission is required.

In addition to the above-mentioned restrictions the following specific restrictions are valid when applying VectoBac G and VectoBac GR aerially:

- Information about the application of the product should be provided to the concerned general public prior to the application.
- Uninvolved third parties shall not stay in or enter treated areas during aerial application.
- The aircraft should be equipped with a professional GPS Guidance system enabling precise application of VectoBac where granted.
- 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.
- Aerial application is only allowed when ground application is not feasible.
- Aerial application is only allowed for areas larger than 0.5 ha.

ANNEX 1. LIST OF STUDIES REVIEWED

Author(s)	Section No / Reference No	Year	Title. Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published	Data Protection Claimed (Yes/No)	Owner
Section 2.1					
Curl, M.G.	IIIB, 2.3/01 Confidential	2013a	Expert statement on the explosive properties of 'VectoBac' G Biological Larvicide granules formulated preparation. TSGE report no. 22-001-17C VectoBac G EXP. Non-GLP, unpublished	Y	Valent Biosciences
Curl, M.G.	IIIB, 2.3/03 Confidential	2013b	Expert statement on the oxidising properties of 'VectoBac' G Larvicide granules formulated preparation. TSGE report no. 22-001-17C VectoBac G OXP. Non-GLP, unpublished	Y	Valent Biosciences
Curl, M.G.	IIIB, 2.4/01 Confidential	2013c	Expert statement on the flammability of 'VectoBac' G Larvicide granulse formulated preparation. TSGE report no. 22-001-17C VectoBac G FLM. Non-GLP, unpublished	Y	Valent Biosciences
Curl, M.G.	IIIB, 2.3/02 Confidential	2013d	Expert statement on the explosive properties of 'VectoBac' GR Biological Larvicide granules formulated preparation. TSGE report no. 22-001-17C VectoBac GR EXP. Non-GLP, unpublished	Y	Valent Biosciences
Curl, M.G.	IIIB, 2.3/04 Confidential	2013e	Expert statement on the oxidising properties of 'VectoBac' GR Larvicide granules formulated preparation. TSGE report no. 22-001-17C VectoBac GR OXP. Non-GLP, unpublished	Y	Valent Biosciences
Curl, M.G.	IIIB, 2.4/02 Confidential	2013f	Expert statement on the flammability of 'VectoBac' GR Larvicide granules formulated preparation. TSGE report no. 22-001-17C VectoBac GR FLM. Non-GLP, unpublished	Y	Valent Biosciences

Author(s)	Section No / Reference No	Year	Title. Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published	Data Protection Claimed (Yes/No)	Owner
Comb, A.L.	IIIB, 2.1/01 IIIB, 2.2/01 IIIB, 2.5/01 IIIB, 2.7.5/01	2013	VBC-60241 Two Year Storage Stability. Huntingdon Life Sciences Ltd., report no. ZAB0116. GLP, unpublished.	Y	Valent Biosciences
Harding, L.	IIIB, 2.1/02 IIIB, 2.5/02 IIIB, 2.7.5/03 IIIB, 2.10/01	2010	Physical Testing on VBC-60233, a Biological Mosquito Larvicide Granular Product. CEM Analytical Services Ltd., report no.CEMS-4846. GLP, unpublished.	Y	Valent Biosciences
Woolley, A and Mullee, D.	IIIB, 2.7.5/02	2004	ABG-6189: Determination of Dust Content and Friability and Attrition. SafePharm Laboratories, report no. 1438/003. GLP, unpublished.	Y	Valent Biosciences
Section 2.2					
Anon	IIIB, 6.1/05	2009	Evaluate initial control and residual efficacy of 5, 10 , 15 and 20 Kg VectoBac G per hectare against Culex sp. in polluted water Trial 2009RFUSC004 (= 2009HKOTT005a) Non-GLP, Unpublished	Y	Valent Biosciences
Anon	IIIB, 6.1/06	2010	Evaluation of the initial mortality of aerial applied VectoBac G for the control of the snowmelt mosquitoes Aedes rusticus and Aedes cantans. Trial 2009HKOTT005b Non-GLP, Unpublished	Y	Valent Biosciences
Anon	IIIB, 6.1/07	2009	VactoBac G efficacy test with Mauyama 150 MD motor sprayer for ground application Trial 2009HKOTT006 report 1 Non-GLP, Unpublished	Y	Valent Biosciences
Anon	IIIB, 6.1/08	2009	VactoBac G efficacy test with Mauyama 150 MD motor sprayer for ground application Trial 2009HKOTT006 report 2 Non-GLP, Unpublished	Y	Valent Biosciences
Anon	IIIB, 6.1/10	2012	Evaluate efficacy of VBC 60233 and VectoBac G for the control of Aedes vexans, Ochlerotatus sticticus larvae in natural habitats in the Rhone-Alps region/France Trial 2012HKOTT005 report 1 Non-GLP, Unpublished	Y	Valent Biosciences

Author(s)	Section No / Reference No	Year	Title. Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published	Data Protection Claimed (Yes/No)	Owner
Anon	IIIB, 6.1/11	2012	Evaluate efficacy of VBC 60233 and VectoBac G for the control of Aedes vexans, Ochlerotatus sticticus larvae in natural habitats in the Rhone-Alps region/France Trial 2012HKOTT005 report 2 Non-GLP, Unpublished	Y	Valent Biosciences
Anon	IIIB, 6.1/15	2012	Evaluation of VectoBac G and VBC 60233 at rates of 3, 6 and 9 kg/ha for Anopheles spp. control Trial 2012HKOTT010 Non-GLP, Unpublished	Y	Valent Biosciences
Anon	IIIB, 6.1/16	2012	Evaluation of VectoBac G and VBC 60233 at rates of 3, 6 and 9 kg/ha for Anopheles spp. control Trial 2012HKOTT011 Non-GLP, Unpublished	Y	Valent Biosciences
Anon	IIIB, 6.1/17	2013	Evaluation of VectoBac G and VBC 60233 at rates of 3, 6 and 9 kg/ha for Ochlerotatus spp. control Trial 2012HKOTT011 Non-GLP, Unpublished	Y	Valent Biosciences
Anon	IIIB, 6.1/22	2001	Report no 3: Helicopter based spraying of VectoBac G against mosquito larvae in Swedish wetlands. Sweden VB G aerial application June 2001 Unpublished	Y	Valent Biosciences
Becker	IIIB, 6.1/03	2011	Evaluation of VectoBac® G for Control of Aedes spp. in Woodland Pools in Germany Trial 2010PDECH041 Non-GLP, Unpublished	Y	Valent Biosciences
Chouin and Macaud	IIIB, 6.1/09	2013	Comparative efficiency of two Bacillus thuringiensis israelensis strain AM 65-52 based granules formulations at application rates of 3, 6, 9 and 12 kg/ha Trial 2012HKOTT01 Non-GLP, Unpublished	Y	Valent Biosciences
Massone	IIIB, 6.1/14	2011	Evaluation of VectoBac® G in Aerial Application for Control of Aedes caspius and Culex spp. in Rice in Italy Trial 2010PDECH013 Non-GLP, Unpublished	Y	Valent Biosciences

Author(s)	Section No / Reference No	Year	Title. Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published	Data Protection Claimed (Yes/No)	Owner
Ruiz, Kotter and Lucientes	IIIB, 6.1/17	2009	Efficacy evalutions of VectoBac G at 3 and 6 and 9 kg/ha for Ochlerotatus caspius control Trial 2010HKOTT009a Non-GLP, Unpublished	Y	Valent Biosciences
Ruiz, Kotter and Lucientes	IIIB, 6.1/18	2009	Efficacy evalutions of VectoBac G at 4 and 7 and 9 kg/ha for the control of Culex sp. larvae in natural habitats in the Monegros County, Aragon, Spain Trial 2010HKOTT009b Non-GLP, Unpublished	Y	Valent Biosciences
Ruiz, Kotter and Lucientes	IIIB, 6.1/19	2011	Efficacy evaluation of VectoBac G at 4 and 7 and 9kg/ha for the control of *Anopheles spec*. larvae in natural habitats in the Monegros County, Aragon, Spain. Trial 2010HKOTT009b Non-GLP, unpublished	Y	Valent Biosciences
Ruiz, Kotter and Lucientes	IIIB, 6.1/20	2009	Efficacy evalutions of VectoBac G at 4, 8, 12 and 16 kg/ha for the control of Culex sp. larvae in natural habitats in the Monegros County, Aragon, Spain Trial 2010HKOTT008 - 1 Non-GLP, Unpublished	Y	Valent Biosciences
Ruiz, Kotter and Lucientes	IIIB, 6.1/21	2009	Efficacy evalutions of VectoBac G at 4, 8, 12 and 16 kg/ha for the control of Culex sp. larvae in natural habitats in the Monegros County, Aragon, Spain Trial 2010HKOTT008 - 2 Non-GLP, Unpublished	Y	Valent Biosciences
Rydzanicz, DeChant, Becker	IIIB, 6.1/01	2008	Comparison of the efficacy of different granular formulations of bacillus thuringiensis israelensis (strain am65-52) against floodwater mosquitoes in irrigation fields in Wrocław, Poland and floodplains of the rhine river, Germany. JAMCA Manuscript VectoBac KABS (Trial 2) Non-GLP, Unpublished	Y	Valent Biosciences

Author(s)	Section No / Reference No	Year	Title. Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published	Data Protection Claimed (Yes/No)	Owner
Rydzanicz, DeChant, Becker	IIIB, 6.1/04	2008	Comparison of the efficacy of different granular formulations of bacillus thuringiensis israelensis (strain am65-52) against floodwater mosquitoes in irrigation fields in Wrocław, Poland and floodplains of the rhine river, Germany. JAMCA Manuscript VectoBac KABS (Trial 1) Non-GLP, Unpublished	Y	Valent Biosciences
Schonig	IIIB, 6.1/02	2011	Evaluation of VectoBac® G, for Control of Anopehles spp. in Germany Trial 2010PDECH012 Non-GLP, Unpublished	No	Public
Talbalaghi, Ali and DeChant	IIIB, 6.1/12	2004	Evaluation of VectoBac® G in Aerial Application for Control of Mosquito Larvae in Rice Paddies Supporting Tall Rice (> 30 cm) in Piedmont, Italy Trial 2004PDECH033 (Trial 1) Non-GLP, Unpublished	Y	Valent Biosciences
Talbalaghi, Ali and DeChant	IIIB, 6.1/13	2004	Evaluation of VectoBac® G in Aerial Application for Control of Mosquito Larvae in Rice Paddies Supporting Tall Rice (> 30 cm) in Piedmont, Italy Trial 2004PDECH033 (Trial 2) Non-GLP, Unpublished	Y	Valent Biosciences
Section 2.5					
Comb, A.L.	IIIB, 5.1/01	2013	VBC-60241: Two year storage stability. Huntington Life Sceinces LTD., unpublished report no. ZAB0116. GLP, unpublished	Y	Valent Biosciences
Section 2.7					
Kumar,T. Ramachandran, PV and Balakrishna Murthy, P.	IIIB, 7.1.2/01	1997	Acute inhalation toxicity of 'VectoBac' G to Wistar rat; Frederick Institute of Plant Protection and Toxicology, Kancheepuram District, Tamil Nadu, India. Report No. 4667 GLP. Unpublished	Y	Valent Biosciences
Ravikrishnan, R and Balakrishna Murthy, P.	IIIB, 7.2.1/01	1997a	Primary skin irritation of 'VectoBac' G in rabbit; Frederick Institute of Plant Protection and Toxicology, Kancheepuram District, Tamil Nadu, India Report No. 4619 GLP. Unpublished	Y	Valent Biosciences

Author(s)	Section No / Reference No	Year	Title. Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published	Data Protection Claimed (Yes/No)	Owner
Ravikrishnan, R and Balakrishna Murthy, P.	IIIB, 7.2.2/01	1997b	Irritation of VectoBac G to mucus membrane in rabbit; Frederick Institute of Plant Protection and Toxicology, Kancheepuram District, Tamil Nadu, India Report No. 4620 GLP. Unpublished	Y	Valent Biosciences
Ravikrishnan, R. Ramachandran, PV and Balakrishna Murthy, P.	IIIB, 7.1.1/01	1997a	Acute oral toxicity of 'VectoBac' G to Wistar rat; Frederick Institute of Plant Protection and Toxicology, Kancheepuram District, Tamil Nadu, India. Report No. 4616 GLP. Unpublished	Y	Valent Biosciences
Ravikrishnan, R. Ramachandran, PV and Balakrishna Murthy, P.	IIIB, 7.1.1/02	1997b	Acute oral toxicity of 'VectoBac' G to Swiss albino mice; Frederick Institute of Plant Protection and Toxicology, Kancheepuram District, Tamil Nadu, India Report No. 4617 GLP. Unpublished	Y	Valent Biosciences
Ravikrishnan, R. Ramachandran, PV and Balakrishna Murthy, P.	IIIB, 7.2.3/01	1997c	Allergy and skin sensitisation potential of VectoBac G in guinea pigs; Frederick Institute of Plant Protection and Toxicology, Kancheepuram District, Tamil Nadu, India. Report No. 4624 GLP. Unpublished	Y	Valent Biosciences

Additional references

From open literature, cited in the report, and evaluated by KemI

Anonymous. 2010. VectoBac G, Product Assessment Report: Swedish Chemicals Agency. Dnr F-3422-221-09.

Hansen, B.M., Damgaard, P.H., Eilenberg, J., Pedersen, J.C. 1996. *Bacillus thuringiensis. Ecology and Environmental Effects of Its Use for Microbial Pest Control.* . Denmark : Danish Environmental Protection Agency. Project no.316.

Glare, T.R., O'Callaghan, M. 2000. *Bacillus thuringiensis*: Biology, Ecology and Safety. John Wiley, N.Y.

Lacey, L.A., Merritt, R.W. 2003. The safety of bacterial microbial agents used for black fly and mosquito control in aquatic environments. . [bokförf.] H M T. Hajeck, A E. Hokkanen. *Environmental impact of microbial insecticides. Need and methods for risk assessment.* u.o. : Kluwe Academic Pub, 2003, ss. 51-167.

Menon, A.S., De Mestral, J. 1985. Survival of *Bacillus thurigiensis* var. *kurstaki* in water. *Water Air Soil Pollut*. **25**: 265-274.

Duchet, C., Larroque, M., Caquet, T.H., Franquet, E., Lagneau, C., Lagadic, L. 2008. Effects of spinosad and *Bacillus thuringiensis* on a natural population of *Daphnia pulex* in field microcosms. *Chemosphere.* 2008, **74**:70-77.

Duchet, C., Inafuku, M.M., Caquet, T.H., Larroque, M., Franquet, E. Lagneau, C. Lagadic, L. 2011. Chitobiase activity as an indicator of altered survival. Growth and reproduction in *Daphnia magna* (Crustacea: Cladocera) exposed to spinosad and diflubenzuron. *Ecotox Environ Safety*, **74**: 800-810.

Vaishnav, D.D., Andersson, R. 1995. Uptake and lossof Bacillus thuringiensis var. israelensis by daphnia magna in laboratory exposure. *Environmental Toxicology and Chemistry*, **14**:763-766.

Ref-MS information to the reader:	Besides submitted studies and cited, the overall evaluation of VectoBac is based on data from published peer-review articles, and reports reviewing previous use in Sweden and other countries. Of particular interest is the PAR from the previous authorisation in Sweden, containing an extensive literature review, included in the reference list above (Anonymous, 2010).

ANNEX 2. METHOD TO MEASURE THE BIOPOTENCY

Method

The assay was based on the quantal dose response of 3 day old Aedes aegypti mosquito larvae to the test substance. The percentage mortality response was analyzed by weighted Probit analysis and was expressed as potency (in International Toxic Units (ITU per mg)) relative to a reference substance.

Reference substance

The reference standard containing *Bacillus thuringiensis* subspecies *israelensis* was stored frozen (approximately -10 to -30°C) prior to use. Individual vials were stored in a desiccator at room temperature for up to one month while in use.

Procedure

The appropriate amount of test substance or reference substance was transferred to a glass bottle. An aliquot (100 mL) of deionised water was added. The contents were then mixed on a shaker for approximately 20 minutes. Portions of the initial stock suspensions were diluted with de-ionised water to produce the final stock suspensions.

The final test concentrations were then prepared. The following table shows the amounts of test substance/reference substance final stock suspension and deionised water which were added to each cup to achieve the test concentrations:

Concentration No.	Deionised water (ml)	Final stock (ml)	Additional deionised water (ml)	Final volume (ml)
1	90	10	0	100
2	90	8.5	1.5	100
3	90	7	3	100
4	90	5.5	4.5	100
5	90	4	6	100
6	90	2.5	7.7	100
Untreated check	90	0	10	100

Four ounce waxed paper cups were used as containers. Each cup was filled 90 mL of deionised water and infested with 20 larvae. After all of the cups had then been treated with the appropriate concentrations of test substance and reference substance, they were covered with wax paper to reduce evaporation. For each test replication, there were 6 cups for each concentration number.

The cups were then placed in an incubator maintained at 28 ± 2°C, 50 ± 15% relative humidity and a 12:12 light:dark cycle for a period of 17 to 20 hours.

After the incubation period, the samples were examined and the number of surviving larvae counted.

The entire test procedure was replicated four times for each lot at each timepoint.

The highest final test concentration (FTC) for the test substance was determined in terms of mass per volume according to the following equation:

TS highest FTC (µg/mL) = [Assigned potency of RS (ITU/mg) / Theoretical potency of TS(ITU/mg)] * Highest FTC of RS (µg/mL)

where, TS = test substance RS = reference substance

Subsequent FTC's are calculated based on the amount of final stock suspension dispensed in the test cups.

If needed, the test substance FTC could be further adjusted based upon preliminary bioassay results.

The test substance potency was then determined for each replication by estimating the LC_{50} using a suitable Probit analysis program and then using the following equation:

TS potency (ITU/mg) = [Estimated LC50 of RS / Estimated LC_{50} of TS RS] * potency (ITU/mg)

where, the LC_{50} is the concentration that produces 50% larval mortality.

Ref-MS information to the reader:	The method is taken from study IIIB, 2.2.1-1.