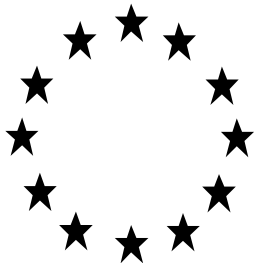
Directive 98/8/EC concerning the placing biocidal products on the market

Inclusion of active substances in Annex I or IA to Directive 98/8/EC

Assessment Report[i]



Bacillus thuringiensis subsp. *israelensis* Serotype H-14
Strain AM65-52

Product-type 18

(Insecticide)

Rapporteur Member State: ITALY

***Bacillus thuringiensis* subsp. *israelensis* – Strain AM65-52 (PT 18)**

Assessment report

Finalised in the Standing Committee on Biocidal Products at its meeting on 15 February 2010 in view of its inclusion in Annex I or IA to Directive 98/8/EC

CONTENTS

1. STATEMENT OF SUBJECT MATTER AND PURPOSE

1.1. Procedure followed

This assessment report has been established as a result of the evaluation of *Bacillus thuringiensis* subsp. *israelensis* – Strain AM65-52 as product-type 18 (Insecticide), carried out in the context of the work programme for the review of existing active substances provided for in Article 16(2) of Directive 98/8/EC concerning the placing of biocidal products on the market[1], with a view to the possible inclusion of this substance into Annex I or IA to the Directive.

Bacillus thuringiensis subsp. *israelensis* – Strain AM65-52 [general CAS N° for Bt's 68038-71-1]) was notified as an existing active substance, by Sumitomo Chemical Agr. Europe SAS (in representation of Valent BioSciences Corporation), hereafter referred to as the applicant, in product-type 18.

Regulation (EC) No 1451/2007 of 4 December 2007,[2] which has repealed and replaced Commission Regulation (EC) No 2032/2003 of 4 November 2003,[3] lays down the detailed rules for the evaluation of dossiers and for the decision-making process in order to include or not an existing active substance into Annex I or IA to the Directive.

In accordance with the provisions of Article 5(2) of Regulation (EC) No 2032/2003, Italy was designated as Rapporteur Member State to carry out the assessment on the basis of the dossier submitted by the applicant. The deadline for submission of a complete dossier for *Bacillus thuringiensis* subsp. *Israelensis* – Strain AM65-52 as an active substance in Product Type 18 was 30-4-2006, in accordance with Annex V of Regulation (EC) No 2032/2003.

On 30-4-2006, Italian competent authorities received a dossier from the applicant. The Rapporteur Member State accepted the dossier as complete for the purpose of the evaluation on 2-11-2006.

On 21-10 2008, the Rapporteur Member State submitted, in accordance with the provisions of Article 14(4) and (6) of Regulation (EC) No 1451/2007, to the Commission and the applicant a copy of the evaluation report, hereafter referred to as the competent authority report. The Commission made the report available to all Member States by electronic means on 22-10-2008. The competent authority report included a recommendation for the inclusion of *Bacillus thuringiensis* subsp. *Israelensis* – Strain AM65-52 in Annex I to the Directive for PT 18.

In accordance with Article 16 of Regulation (EC) No 1451/2007, the Commission made the competent authority report publicly available by electronic means on 5-11-2008. This report did

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1 Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998 concerning the placing biocidal products on the market. OJ L 123, 24.4.98, p.1

2 Commission Regulation (EC) No 1451/2007 of 4 December 2007 on the second phase of the 10-year work programme referred to in Article 16(2) of Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market. OJ L 325, 11.12.2007, p. 3

3 Commission Regulation (EC) No 2032/2003 of 4 November 2003 on the second phase of the 10-year work programme referred to in Article 16(2) of Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market and amending Regulation (EC) No 1896/2000. OJ L 307, 24.11.2003, p. 1

not include such information that was to be treated as confidential in accordance with Article 19 of Directive 98/8/EC.
In order to review the competent authority report and the comments received on it, consultations of technical experts from all Member States (peer review) were organised by the Commission. Revisions agreed upon were presented at technical and competent authority meetings and the competent authority report was amended accordingly.

On the basis of the final competent authority report, the Commission proposed the inclusion of *Bacillus thuringiensis* subsp. *israelensis* – Strain AM65-52 in Annex I to Directive 98/8/EC and consulted the Standing Committee on Biocidal Product on - 6-5-2011
In accordance with Article 15(4) of Regulation (EC) No 1451/2007, the present assessment report contains the conclusions of the Standing Committee on Biocidal Products, as finalised during its meeting held on—6-5-2011.

1.2. Purpose of the assessment report

This assessment report has been developed and finalised in support of the decision to include *Bacillus thuringiensis* subsp. *israelensis* – Strain AM65-52 in Annex I to Directive 98/8/EC for product-type 18. The aim of the assessment report is to facilitate the authorisation /registration in Member States of individual biocidal products in product-type 18 that contain *Bacillus thuringiensis* subsp. *israelensis* – Strain AM65-52. In their evaluation, Member States shall apply the provisions of Directive 98/8/EC, in particular the provisions of Article 5 as well as the common principles laid down in Annex VI.
For the implementation of the common principles of Annex VI, the content and conclusions of this assessment report, which is available at the Commission website[4], shall be taken into account.
However, where conclusions of this assessment report are based on data protected under the provisions of Directive 98/8/EC, such conclusions may not be used to the benefit of another applicant, unless access to these data has been granted.

1.3. Overall conclusion in the context of Directive 98/8/EC

The overall conclusion from the evaluation is that it may be expected that there are products containing *Bacillus thuringiensis* subsp. *israelensis* – Strain AM65-52 for the product-type 18, which will fulfil the requirements laid down in Article 10(1) and (2) of Directive 98/8/EC. This conclusion is however subject to:

i. compliance with the particular requirements in the following sections of this assessment report,

ii. the implementation of the provisions of Article 5(1) of Directive 98/8/EC, and

iii. the common principles laid down in Annex VI to Directive 98/8/EC.

Furthermore, these conclusions were reached within the framework of the uses that were proposed

4 http://ec.europa.eu/comm/environment/biocides/index.htm

and supported by the applicant (see Appendix II). Extension of the use pattern beyond those described will require an evaluation at product authorisation level in order to establish whether the proposed extensions of use will satisfy the requirements of Article 5(1) and of the common principles laid down in Annex VI to Directive 98/8/EC.

2. OVERALL SUMMARY AND CONCLUSIONS

2.1. Presentation of the Active Substance

2.1.1. Identity, Physico-Chemical and Biological Properties, Methods of Analysis

Bacillus thuringiensis subsp. *israelensis*, Serotype H-14, strain AM65-52 (abbreviated to *Bti* AM65-52) is the biological insecticide active micro-organism of the biocide product 'VectoBac' WG and is manufactured by submerged pure culture fermentation. The technical grade fermentation slurry contains nominally 14% *Bti* AM65-52 fermentation solids, spores, and insecticidal toxins. The formulated product 'VectoBac' WG contains 37.4% of the dried technical grade slurry with low and high limits of 33% and 47% by weight, respectively.

Bti AM65-52 is a Gram positive, spore forming rod-shaped bacterium that produces a crystalline protein inclusion which is toxic to the larvae of some dipteran insects upon ingestion. *Bti* AM65-52 originates from a natural wild strain of the bacteria and has not been genetically modified nor is it the result of a spontaneous or an induced mutation. *Bacillus thuringiensis* subsp *israelensis* is a common naturally occurring micro-organism with worldwide distribution. The species has been detected both in soil and on insects and plants and could be indigenous to intended areas of application. Identification of the a.s. at strain level has been performed by genomotyping, i.e. analysis of bacteria by comparison of their genomes using microarrays (Lucchini *et al*., 2001). The technique allows to observe gene complement differences between strains of the same subspecies, and the identification to strain level is considered satisfactory.

The mode of action of *Bti* AM65-52 results from toxic proteins, as protoxins, contained in parasporal crystals. The crystals are taken up by the target insect larvae via ingestion. Under the alkali conditions present in the larvae gut and for the action of gut proteases the crystal dissolves releasing the active protein δ-endotoxins that induce disintegration of the gut epithelium

Bacillus thuringiensis spores are resistant to desiccation, heat, ultraviolet irradiation and other factors such as chemical disinfectants, some antibiotics and can survive in environments protected from sunlight (e.g. soil) for many months. Transfer of antibiotic resistance to other bacteria is possible. However, being resistance genes mostly chromosomally encoded, the transfer is unlikely. Degradation of the insect toxins and vegetative cells, however, is more rapid and is generally measured in days in most situations. In water, *Bacillus thuringiensis* is rarely detected for more than a few days after application and on foliage *Bacillus thuringiensis* and its associated insect toxins do not persist.

A methodology for the identification of *Bti* AM65-52 in the technical grade micro-organism has been developed, mainly based upon genomotyping. Details of these methods are provided in the additional information provided by the Applicant.

Methods of analysis in food and feed are not considered relevant since the biocidal use of *Bti* AM65-52 is for the control of larvae of mosquitoes and black flies in water habitats and larvae of filter fly midges in sewage treatment plants. *Bti* AM65-52 is not used on clean purified drinking water .

Vegetative cells of *Bti* have a limited survival time in the environment and spores do not germinate readily, making it highly unlikely that *Bti* will multiply and colonise areas of intended use above levels that may occur naturally. Since *Bti* is a naturally occurring organism, methods for determining residues of *Bti* AM65-52 in

environmental compartments are not considered necessary.
The methods presented for the analysis of *Bti* AM65-52 are considered to be appropriate for determination of *Bti* AM65-52 in the formulated product. The methods contain information confidential to Valent BioSciences and are shown in the confidential attachment.

2.1.2. *Intended Uses and Efficacy*

Bti AM65-52 is a biological larvicide. The intended field of use is Pest Control (Main Group 3) under Product Type 18 (insecticide).
The biocidal use of *Bti* AM65-52 is for the control of larvae of mosquitoes and black flies in water habitats and larvae of filter fly midges in sewage treatment plants.
The assessment of the biocidal activity of the active substance demonstrates that it has a sufficient level of efficacy against the target organisms and the evaluation of the summary data provided in support of the efficacy of the accompanying product, establishes that the product may be expected to be efficacious.
In the laboratory, resistance has been developed for several insects to the *Bacillus thuringiensis* subspecies *kurstaki, aizawai, entomocidus* and *tenebrionis* (*san diego*) and to 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.
Bti AM65-52 is a Gram positive, spore forming rod-shaped bacterium that produces a crystalline protein inclusion which is toxic to larvae of some dipteran insects upon ingestion. The mode of action of *Bti* AM65-52 results from toxic proteins contained in the crystalline protein inclusion. The crystals are taken up by the target insect larvae via ingestion and under the alkali conditions present in the larvae gut and for the action of gut proteases the crystal dissolves releasing the active protein δ-endotoxins (Cry4Aa1, Cry4Ba1, Cry10Aa1, Cry11Aa1 and Cyt1Aa1) that induce disintegration of the larvae gut epithelium and consequent death of the larvae. The unbalanced or prevailing presence of some of the endotoxins with respect to the overall number cannot be excluded. However, their quantification appears still difficult because the relative amounts will depend from insect gut conditions.
Information is available from a series of field experiments to show that 'VectoBac' WG is effective under a range of conditions against a variety of mosquito species including; *Aedes* spp.(*Aedes albopictus, Aedes aegypti, Aedes notoscriptus, Aedes vexans* and *Aedes vigilax), Culex spp.*(*Culex annulirostris, Culex quinquefasciatus, Culex sitiens*) The tests were performed at rates up to 500 g/ha ($9x10^{12}$ CFU/ha;$1.5x10^{9}$ ITU/ha), with the target species present between the 1st and early 4th instar larval growth stage. Mortality greater than 95% of the control was observed after 48 hours.
Information is available from field experiments to show that *Bti* AM65-52 is highly effective against black flies (*Simulidae*) and a range of filter flies including; *Sylvicola spp, Metriocnemus hygropetricus, Orthocladius fuscinanus, Psychoda alternata* and *P. severini*. Although not conducted using 'VectoBac' WG, these studies are considered representative of the likely efficacy of 'VectoBac' WG against the target pest. Different formulations of *Bti* AM65-52 may be better suited to these organisms.
Although some of the presented documents on efficacy are not scientifically acceptable (see comments on single documents in section III.6B), VectoBac WG and other Bti strains have been

used successfully for several decades against many species of mosquito and blackfly larvae and larvae of filter fly midges.
In conclusion, 'VectoBac' WG is sufficiently effective against larvae of mosquito and black flies and larvae of filter fly midges and the results of the efficacy studies support the label recommendations.

'VectoBac' WG is not an adulticide and application when larvae are present up to the early 4th instar growth stage is necessary for effective control.

In addition, in order to facilitate the work of Member States in granting or reviewing authorisations, and to apply adequately the provisions of Article 5(1) of Directive 98/8/EC and the common principles laid down in Annex VI of that Directive, the intended uses of the substance, as identified during the evaluation process, are listed in Appendix II.

2.1.3. Classification and Labelling of the active substance

Hazard symbol:	
Risk phrases	.
Safety phrases	S2, S13, S20/21, S24, S28, S37

The summary of findings from laboratory studies, submitted literature and regulatory reviews, in conjunction with the medical surveillance reports from production areas is that *Bacillus thuringiensis* subsp. *israelensis*, Serotype H-14, Strain AM65-52 poses limited risk to human health, related only to the possibility to induce sensitization, based on the results obtained on animal models. Acute intravenous administration to rats of approximately 10^7 CFU resulted in no treatment related toxicity and no evidence of pathogenicity. Intraperitoneal injection of 10^6, 10^7 or 10^8 CFU/g to mice resulted in no signs of toxicity or pathogenicity. None of the studies with Bti AM65-52 showed signs of infectivity or pathogenicity by routes of maximum challenge. This is consistent with published study findings and regulatory reviews of other *Bt*, strains.
Bacillus thuringiensis subsp. *israelensis*, Serotype H-14, Strain AM65-52 is therefore unlikely to cause human disease and can be classified as a Group 1 biological agent according to Article 2 of Directive 2000/54/EC.

2.2. Summary of the Risk Assessment

2.2.1. Human Health Risk Assessment

2.2.1.1. Hazard identification and Effects assessment

Toxicity, irritancy and sensitisation

The basic acute studies confirmed *Bti* AM65-52 to be of low oral, dermal and inhalation toxicity. The results were typically:

The median lethal oral dose level (LD_{50}) of *Bacillus thuringiensis* in rats was determined to be greater than 5000 mg/kg. The median lethal dermal dose level (LD_{50}) of *Bacillus thuringiensis* in rabbits was found to be greater than 5000 mg/kg. Rats were exposed to the undiluted bacterial spores, presented as an aerosol for four hours, at the maximum attainable chamber concentration of 2.84 mg/L. The test aerosol produced no deaths, no clinical signs other than procedurally induced changes in behaviour on the day of exposure. Bodyweights were unaffected by treatment. A concentration of 2.84 mg/L was considered to be a NOAEL and the acute LC_{50} therefore exceeded 2.84 mg/L.

Studies of dermal irritation, including single administration to abraded or non-abraded test sites consistently indicated the test material had the potential to elicit no more than mild skin irritation - reactions not exceeding well defined erythema in rabbits.

The available eye irritation study showed *Bti* AM65-52 is not an ocular irritant. Investigations in rinsed and non-rinsed eyes showed the majority of irritation reactions were reversible (i.e. the eyes were overtly normal) within 72 hours. The mean scores for the 24, 48 and 72 hour assessments did not exceed the criteria for labelling and classification as an ocular irritant in accordance with Commission Directive 2001/59/EC.

Two assays for skin sensitisation were available, a conventional guinea pig test conducted to the Beuhler protocol design which gave a mild sensitising response and a M-K test with the formulation which produced no sensitization.

There have been no medical surveillance abnormalities or reports to the Occupational Health Services by employees at the manufacturing site to date regarding health related or other adverse reactions.

A number of acute administration studies, complying with the maximum challenge approach, were completed to investigate possible infectivity or pathogenicity via oral, intravenous or intratracheal routes of administration. Acute oral administration of *B. thuringiensis* to rats at approximately 10^8 colony forming units (CFU) per animal resulted in no deaths or adverse clinical signs. The test compound was found to be neither toxic nor pathogenic. None of the rats died and no adverse clinical signs were apparent. *Bti* AM65-52 was neither toxic nor pathogenic to rats. .

Acute intratracheal instillation of *B. thuringiensis* var. *israelensis* to rats at approximately 10^8 CFU of 'VectoBac' technical material resulted in signs of toxicity during the first two days following dosing. Signs observed included ruffled coat, lethargy, and effects on body posture, respiration and locomotor activity but these had all resolved by Day 3. There was no evidence of pathogenicity and no mortality during the study. However the effects were considered to be due to the presence of foreign material in the lungs rather than an infective process.

In a second study by the intratracheal route, *Bti* AM65-52 administered to male rats at approximately 8 x 10^7 CFU/ml resulted in no deaths or adverse clinical signs. Treated rats were slightly less efficient at food conversion than controls, showing less initial weight gain and increased food consumption in the first 24 hours following dosing. Subsequently these parameters were similar for control and treated groups. The test compound was found to be neither toxic nor pathogenic to rats.

Acute intravenous administration to rats of approximately 10^7 CFU resulted in no treatment related toxicity and no evidence of pathogenicity. Intraperitoneal injection of 10^6, 10^7 or 10^8 CFU/g to mice resulted in no signs of toxicity or pathogenicity.

Genotoxicity

The requirement for genotoxicity testing of microbials should be based on the characteristics of the micro-organism in question, their infectivity potential of mammalian cells, the known natural occurrence and previous human exposure to the micro-organism, and the genotoxicity potential of toxins and metabolic by-products. The guidelines currently in place for genotoxicity testing have been developed to test chemicals. The use of these guidelines poses certain problems when testing microbials. It is recognized that the physicochemical properties of a substance (e.g., volatility, pH, solubility, stability, its purity, etc.) can sometimes make standard test conditions inappropriate. This becomes even more apparent as one considers microbial organisms. Standard mutagenicity and genotoxicity assays are not considered appropriate for many living micro-organisms 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.

Short-term toxicity

Short-term toxicity investigations were limited to two studies completed with 'VectoBac' 12 AS, a formulation containing 10^6 *Bti* AM65-52 spores/mL. In the first study, groups of dogs were dosed for 90 consecutive days, resulting in no mortality or treatment-related adverse clinical signs. Pathological examination and terminal necropsy revealed no effects of 'VectoBac' 12 AS administration. No evidence for subacute toxicity of *Bti* AM65-52 was found in the dog dosed at circa 10^6 *Bti* spores/mL.

In the second study, groups of four male and four female rats were repeatedly exposed to a test atmosphere. 'VectoBac' 12 AS was not found to be toxic to rats by the inhalation route when repeatedly administered at up to 1.8 x 10^6 spores/L air. There were no mortalities during the study, no treatment-related adverse clinical signs of reaction and no changes in various in-life parameters. Post-mortem examinations revealed no treatment-related changes.

Summary of mammalian toxicity

A summary of toxicity related to metabolites of *Bti* – or, more specifically, to the four protein complex involved in parasporal body toxicity, is presented in section IIIA 5.3. Further discussion in published literature indicates the highly species specific nature of the *Bti* δ-endotoxin, the lack of toxic effects in warm-blooded organisms and the lack of activation in the non-alkaline gut environment of mammals.

In a range of toxicological studies, completed using *Bti*, experimental infection of mice, rats, guinea pigs and rabbits was attempted by various routes. Single and repeat administration tests revealed an absence of acute or prolonged toxicity at doses of approximately 10^7 to 10^8 bacteria per animal. There were no indications of anaphylaxis in guinea pigs and repeated passage through mice induced no virulent response. Repeat administration of a dose in the order of 10^{11} or 10^{12} bacteria per rat/mouse for three weeks resulted in no pathogenicity. In none of these tests was there evidence of pathological symptoms, disease or mortality. Behavior and weight gain were unaffected by treatment and necropsy revealed no macroscopic effects. The re-isolation

tests for various organs were negative. It was conclude that *Bti* was well tolerated by the test species used, showed no propensity to multiply within the host and was rapidly eliminated without causing adverse effects. *Bti* was confirmed to be innocuous.
An assessment of the health effects of Bti on operators involved in the fermentation process and other persons likely to be exposed to the material is presented as a summary of medical surveillance. The Medical Director responsible for the plant confirmed no abnormalities and no human health related or other adverse reactions to Bti.
Discussion of human infection in relation to Bacillus species is presented in IIIA 5.1.4 with details of a number of patients examined for the presence of Bacillus species in a general hospital. The overall results indicate that *B. thuringiensis* may be responsible for opportunistic infections and that the possibility of a human infection with *B. thuringiensis* is limited only to severe immunocompromised patients. There are no indications that Bti AM65-52 is involved in human pathogenicity, infectivity or toxicity.
The overall assessment of the acute toxicity/infectivity pathogenicity studies on *Bti* (AM65-52) indicates no evidence of toxicity/infectivity or pathogenicity for the human health.
Bti AM65-52 should be considered as a potential human sensitizer, at concentration above 5,0% w/v, as clearly demonstrate in a experimental test study on guinea pigs, according to the Buehler protocol.

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

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

(1) A potential to cause infection in humans.

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

The safety of *B. thuringiensis* (*Bt*) to mammals has been extensively evaluated with high levels of the entomopathogen administered by various parenteral or oral routes of exposure. There is no evidence to lead to a conclusion that the limited exposures following use of the biocidal product could result in a direct toxic effect in humans.
However, the *Bacillus* genus contains the virulent mammalian pathogen *B. anthracis*, and any assessment of *Bti* AM65-52 should include an assessment of the potential for the bacterium to cause infection in humans exposed to the biocidal product. Equally, the endotoxin produced by *Bti* AM65-52 is immunologically similar to the enterotoxin produced by *B. cereus* which is known to cause diarrhoeal food poisoning. Nevertheless, the producer has shown that no enterotoxins are present in the manufactured product.
The ability of *Bti* AM65-52 to remain viable in mammalian tissue may lead to detection in humans, particularly in environments where the microbial agent is used for insect vector control. In addition, the ubiquitous nature of *B. thuringiensis* subsp. *israelensis* (*Bti*) and its persistence has meant it has been identified as present in infections following traumatic wounding, although no confirmation that *Bti* has been causative in the infection process has been established.
There have been no reports of infective activity in cases where humans have been exposed directly (i.e. spraying preparations) to *Bti*. In terms of mammalian infection, the specific toxicity of the parasporal body is important because it is not activated in mammals. Clearance rates may

be affected by the presence of vegetative forms in the inoculum. The toxicity of the alkali-solubilised crystal δ-endotoxin of *Bti* is only relevant to the insect GI tract because it is not activated in the acidic conditions of the mammalian intestine. Therefore, the risk of *Bti* AM65-52 causing true infectious disease in mammals, including humans, is considered to be negligible.
Animal testing using a variety of conventional toxicity tests and a range of maximum challenge protocols has been completed to confirm that *Bti* has no adverse effects. Rats fed 2 x 10^{12} viable spores per kg bodyweight showed no adverse effects, and human volunteers were fed 3 x 10^{9} spores per day for five consecutive days also without adverse effect (studies reported in 1959).
Bti entered the general circulation following s.c., i.p. or i.c. injection, and was detected in several tissues. The entomopathogen was rapidly cleared from the lungs of rats with no evidence of multiplication to indicate true infectivity. It was shown athymic mice were still capable of clearing the entomopathogen from the body and therefore an intact immune system was not required for successful clearance. However, athymic mice had higher levels in the spleen than euthymic mice.
Acute intratracheal instillation of *Bti* to rats at *ca* 10^{8} CFU of 'Vectobac' technical material resulted in signs of toxicity during the first two days following dosing, but no evidence of pathogenicity or mortality. Acute intravenous administration to rats of *ca* 10^{7} CFU resulted in no treatment related toxicity and no evidence of pathogenicity.
This was also the case with mice dosed by intraperitoneal injection of 10^{6}, 10^{7} or 10^{8} CFU/g. No evidence for sub-acute toxicity of *Bti* AM65-52 was found in the dog dosed at *ca* 10^{6} *Bti* spores/mL for 90 days and there were no indications of treatment-related toxicity among rats dosed for 14 days by inhalation exposure at up to 1.84 x 10^{6} spores/L air/day.
Cell culture studies are required for viruses and viroids or specific bacteria and protozoa with intracellular replication. This is not applicable to *Bti* AM65-52 which does not replicate in warm-blooded organisms.
The *Bti* δ-endotoxin consists of a four protein complex and is specifically toxic to insects, as it requires a very high pH 10 for activation. The lack of toxic effects in warm-blooded organisms and the lack of activation in the non-alkaline gut environments of mammals results in no adverse effect of the material in the context of human health.
An assessment of the health effects of *Bti* on operators involved in the fermentation process and other staff likely to be exposed to the material confirmed no abnormalities and no human health related or other adverse reactions to *Bti*. However the specific exposure condition are not representative for the Vectobac proposed uses.
An investigation into human infection by the *Bacillus* genus within the confines of a hospital looked at *Bt* presence in post-trauma infection. While the study concluded the presence of *Bt* did not constitute transient bacteraemia, it recognised that strain definition and strain pathogenicity are vital factors in the disease evaluation process. The study concluded that *Bti* AM65-52 is not implicated as a causative agent in human infection.
A study was presented to investigate the hypersensitivity potential of the technical powder product, 'Vectobac', based on *Bti* AM65-52, using the Buehler method. The results of this study indicate that Technical Powder VectoBac (Code 43494) administered as a 50% w/v formulation in distilled water during induction and as a 5% w/v formulation in distilled water during primary challenge, does produce dermal sensitization in the guinea pig. The formulated product VectoBac WG, under a Maximization test, was not considered a sensitizer.

Several *Bt* products including Vectobac WG have been in use for several decades, according to the manufacturer, with no severe findings reported. However data show that some adverse effects occur following direct human exposure especially during and after spraying in general population, but the amount and the relevance of the symptoms observed were not coherent among studies. Many of the effects observed were related to respiratory distress as well as skin reactions supporting the hypothesis that the exposure to commercial products based on *Bt* could possibly lead to sensitization/allergenicity reactions.
A study on humans showed that after exposure it is possible to observe vegetative *Bti* AM65-52 presence in samples, followed by clearance (which occurs after several days or weeks), without acute adverse effects.

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

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

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

2.2.1.2. Exposure assessment and risk characterisation

Human health risk for professional users

'VectoBac' WG poses potentially minimal risk to human health and the risks to professional workers through either manufacture or use of the active micro-organism or formulated product are limited if PPE are used and the indication of use strictly followed .
The potential for professional workers to be exposed to 'VectoBac' WG during use is summarized below:

Inhalation exposure

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

Oral exposure

If PPE are worn correctly 'VectoBac' WG is unlikely to reach the mouth of professional users.

Dermal exposure

'VectoBac' WG is a WG formulation. Professional users could be exposed dermally during mixing/loading of the spray solution 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.

Professional 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 users wearing protective equipment are permitted in areas being treated.

Human health risk from indirect exposure as a result of use

VectoBac' WG' poses minimal risk to human health and the risks to non-users through indirect exposure are negligible if the biocide is not used by aerial spray or on food and water intended for human uses.

2.2.2. Environmental Risk Assessment

2.2.2.1. Fate and distribution in the environment

Bacillus thuringiensis (*Bt*) has been isolated worldwide from a range of habitats. In soil, the number of *Bt* spores has been found to vary between less than $2x10^2$ to $5x10^4$/g soil (P.A.W. Martin, 1991) and can persist for 1-2 years.

Pedo climatic conditions are likely to affect persistence, e.g. organic matter content, pH, soil texture, solar radiation etc. The long survival of spores in soils has been confirmed by Vettori et al. (2003). They showed *Btk* and its toxin introduced into soils in sprays can persist for long periods (at least 88 months for *Btk* and at least 28 months for its toxin).

Although *Bt* bacteria generally represent an indigenous part of the soil microbiota community (De Respinis *et al*., 2006; Vettori et al., 2003) they do not compete aggressively with other soil micro-organisms (West et al., 1984; Akiba, 1986) and, as result of degradation of vegetative cells and poor germination of spores, are not adapted to survive as "active" members of the soil microbial community.

As a general figure, the occurrence of *B.thuringiensis* subsp. *israelensis* (*Bti*) in soil accounts for about 20% of *Bt* serotypes (Martin and Travers, 1989) .Experimentally determined half-lives in soil are usually in the range of 100-200 days (Hansen et al., 1996). In a field soil, Pedersen et al. (1995) found both a long-term persistency of *Bt*k DMU67R spores, with a half-life of 120 days, and an extremely short half-life in the phylloplane.

Supplementing soil with nutrients, or autoclaving, stimulates the growth, while decreasing the pH from 7.3 to 5.2 has the opposite effect. *Bt* subsp. *aizawai* grows faster and survived better in wet soils (0,-0.1 Mpa) than in dry soils (West et al., 1985).

In soil, the persistence of protein-crystals, assessed by bioassay of insecticidal activity, fall rapidly as consequence of degradation by microorganisms and adsorption onto soil particles. Pruett et al. (1980) found that the death of spores and reduction in potency follow exponential

curves typical of decay processes, with potency falling much faster than viable spores. Bioassay data showed that at no time would the observed death rate of the spores accounts for the observed fall in potency. The insecticidal activity half-life has been calculated (West, 1984) in the range 2.7-5.2 days in absence and following the addition of an organic supplement, respectively.

In experiments in Japan, Akiba (1991, summarized by Goodyear, 2005) found that under artificially and naturally irrigated conditions, there was no translocation of sprayed *Bt* into the soil down to a depth of 10 cm. Additionally, adsorption and binding of spores, protoxins and toxins from *Btk* have been demonstrated to occur readily, rapidly and strongly onto the clay fraction and clay humic acid complexes of soils (Venkateswerlu and Stotzky 1992, summarized in Goodyear, 2005; Tapp and Stotzky, 1995; Crecchio and Stotzky, 1998; Crecchio and Stotzky, 2001).

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

Menon and De Mestral (1985) investigated over a period of 70 days in the laboratory the survival of *Btk* viable cells in four types of water: filtered-distilled, tap, lake and sea water. A similar declining trend in *Btk* survival was seen in distilled and tap water where approximately 50 % of the original cell population died off rather rapidly during the first 20 days following inoculation. *Btk* was found to be far more persistent in fresh water than in sea water, generally considered bactericidal to non-marine bacteria (Pramer et al., 1963, cited in Menon and De Mestral, 1985), whereas lake water (approximately half-life 50d) contains a higher concentration of available nutrients favourable to *Btk* survival.

Based on the findings of several studies reported in the scientific literature that have investigated the persistence of *Bti* in water (Mulla et al., 1985; Beehler et al., 1991; Hougard et al., 1995; all cited in Glare and O'Callaghan, 2000), larvicidal activity of Bti disappears within 1-4 weeks.

Potential atmospheric exposure of *Bti* AM65-52 may occur following commercial applications. However, a rapid degradation in air is assumed since inactivation by solar radiation is a very important factor causing loss of activity and degradation of bacteria spores and δ-endotoxin crystals in the field environment (Griego and Spence, 1978 Myasnik et al., 2001; Pusztai et al., 1991). Following an aerial spray program, *Btk* concentrations in the air showed an initial half-life (10-hour period from start of spraying) of 3.3 hours. The overall half-life determined during the nine-day monitoring period was 2.4 days, (Teschke *et al.*, 2001).

It can be conclude that *Bti* vegetative cells and insecticidal toxins of *Bti* have a limited survival time in the environment and the spores do not germinate readily, making it unlikely that *Bti* AM65-52 will multiply and colonize areas of intended use above levels that may occur naturally.

As metrics, NOED (not observed effect density) has been used hereafter, considering that the values are referred to a microorganism and not to a chemical

2.2.2.2. Effects assessment

Aquatic compartment

Toxicity to Fish

Bti AM65-52 is not considered to be acutely toxic to fish; there is no evidence of significant effects following long-term exposure. A summary of the toxicity values for fish exposed to *Bti* AM65-52 is presented in Table 1.

Table 1 Summary of the fish toxicity data for *Bti* AM65-52

Study type	Species	Exposure		Result			Reference
			Endpoint	CFU/L-CFU/g	ITU/L-ITU/g	mg MPCA/L	
Acute	*Oncorhynchus mykiss*	Static	96-hour LC_{50}	ns	ns	>370	IIIA, 8.2.1-01
	Lepomis macrochirus	Static	96-hour LC_{50}	ns	ns	>600	IIIA, 8.2.1-02
32-day chronic	*Oncorhynchus mykiss*	Semi-static	NOED[a]	1.1x 10^{10c}(aqueous) 1.72 x 10^{10d}(dietary)	$3.7x10^5$ $5.7x10^5$	ns	IIIA, 8.2.1-03
30-day chronic	*Lepomis macrochirus*	Semi-static	NOED[b]	1.2x 10^{10c} 1.31 x 10^{10d}	$4x10^5$ $4.4x10^5$	ns	IIIA, 8.2.1-04
30-day chronic	*Cyprinodon variegatus*	Semi-static	NOED[b]	1.3x 10^{10c} 2.1x 10^{10d}	$4.3x10^5$ $7x10^5$	ns	IIIA, 8.2.1-05

ns – not stated in report

[a] – based on survival, infectivity and pathogenicity, there was a significant effect on growth compared to the control

[b] – based on survival, infectivity, pathogenicity and growth

[c] – measured aqueous concentration (CFU/L)

[d] – measured dietary concentration (CFU/g)

*VectoBac Technical used in chronic toxicity bioassays had a biopotency of $2x10^{11}$ CFU/g MPCA and $6.6x10^3$ ITU/mg MPCA

Toxicity to invertebrates

Bti 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=0.5 g MPCA/L ($1x10^8$ CFU/L; $3.3x10^3$ ITU/L) was observed (although the Applicant suggested a NOEC of 5 mg/l for *Daphnia magna* reproduction test, the application of standard statistical methods accepted at EU level -

Guideline OECD TG211- showed a NOEC of 0.5 mg/l, so that this is proposed value). A summary of the toxicity values for aquatic invertebrates exposed to *Bti* AM65-52 is presented in Table 2.

Table 2 Summary of the aquatic invertebrate toxicity data for *Bti* AM65-52*

Study type	Species	Exposure		Result			Reference
			Endpoint	CFU/L-CFU/g	ITU/L-ITU/g	mg MPCA/L - mg MPCA/kg	
10-days	*Daphnia magna*	Semi-static	10-day LC_{50} NOED/NOEC	>1x10^{10**}	n.s.	>50 50 mg/L	IIIA, 8.2.2-01
21-day reproduction	*Daphnia magna*	Semi-static	NOED/NOEC[a]	1 x 10^{8d}	3.3 x 10^{3}	0.5	IIIA, 8.2.2-02
31-day chronic	Grass shrimp (*Palaemonetes vulgaris)*	Semi-static	NOED/NOEC[d]	2.0 x 10^{10e}	6.6 x 10^{5}	n.s.	IIIA, 8.2.2-03
18-day chronic	Mayfly nymphs (*Hexagenia* sp)	Semi-static	NOED/NOEC[bh]	2.0 x 10^{10f}	n.s.	ns	IIIA, 8.2.2-04
10-day chronic	*Amphiascus minutus*	Static	10-day LC_{50} NOED/NOEC[c]	1x10^{10}	3.3x10^{5}	>50 mg/kg 50 mg/kg	IIIA, 8.2.2-05

ns – not stated in report
[a] – based on adult survival, juvenile production and adult dry weight at day 21
[b] – based on survival, infectivity, pathogenicity and growth
[c] – based on juvenile production
[d] – based on nominal exposure concentration (CFU/L)
[e] – measured dietary concentration (CFU/g)
[f] – measured aqueous concentration (CFU/L)

*Vectobac Technical used in bioassays had a biopotency of 2x10^{11} CFU/g MPCA and 6.6x10^{3} ITU/mg MPCA

** Vectobac Technical used in bioassays had a biopotency of 7.2x10^{10} CFU/g; no ITU content was indicated

Daphnia was the most susceptible among the tested species. In particular, in 21-day chronic toxicity test adverse effects on number of offspring were observed at 5 mg/L (LOEC=5 mg/L; LOED=1x10^{9} CFU/L; LOEC=3.3x10^{4} ITU/L), so that a NOEC of 0.5 mg/L was established.

In addition to these laboratory studies, two field studies were presented, to describe the effects of repeated treatments with *Bti* on non target species. The first paper showed no effects on density and biomass of insects and other benthic macroinvertebrates in three ponds where multiple applications of 'VectoBac'-G were applied. In the second experiment, repeated treatments with Teknar (Bti SA 3A) were applied against black fly larvae so that no detectable non-target effects of Bti application on a wide range of non-target species were observed.
However, as regards long term effects of *Bti* treatments, a study by Hershey *et al*, 1998 in Minnesota wetlands showed as after three years of VectoBac applications the number of non dipteran predators was affected, so that the need for long-term data to evaluate food web effects was expressed. Also Pont et al., 1999; Tilquin et al., 2008 observed some negative effects on repeated treatment with Bti. On the opposite, other papers showed the lack of negative impact on treated ecosystems (Balcer et al., 1999; Schmude et al., 1999; Becker, 2005; Lacey & Merritt, 2002; Lacey, 2007 and, more recently, Lundstrom et al., 2009), so that there are not unambiguous evidences. Until this moment, the risk of human infection with a mosquito-borne pathogen in Europe is not as critical as in other countries (e.g. where malaria problems are dramatic) but it cannot be under evaluated. Sanitary problems for humans and animals related to mosquito bitings are severe, or potentially severe, in many EU regions like recent infection of Chykungunyia virus in Italy (Rezza et al., 2007) or West Nyle Disease virus (Zeller & Schuffeneker, 2004) and Dirofilaria infections (Genchi et al., 2009). In this perspective, mosquito control assumes a strategic importance especially in some areas. Actually, the control is based on reduction of larval populations with larvicides which are distributed in mosquito breeding sites. In urban environments, most part of treatments is carried out inside gully holes, drainage tubes, sewage plants, where biocenosis and trophic chains are relatively simple.

Effects on algal growth

No laboratory studies with algae have been performed according to internationally recognised guidelines. However, a study has been reported (Koskella and Stotzky, 2002) using toxins from *Bti* (25 – 130 kDa) which were purified from 3-5 day old cultures. 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 the cyanobacterium.

Toxicity to aquatic plants

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 from *Bti* AM65-52 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.

Terrestrial compartment

Toxicity to earthworms

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* (Table 3).

Table 3 Effects of Bti AM65 52 *on earthworms

Organism	Study type	Dose applied	Effects	Endpoint	Reference
Eisenia fetida	30-day exposure	1000 mg/kg dry weight soil	No adverse effects	30 day LC_{50} > 1000 mg/kg dry weight soil ($4.8x10^{10}$ CFU/kg d w soil; $8x10^{6}$ ITU/kg d w soil)	8.5-01

* VectoBac Technical used in bioassays had a biopotency of $2x10^{11}$ CFU/g MPCA and $6.6x10^{3}$ ITU

A field study has been reported on *Bt* subsp. *kurstaki* (Benz and Altwegg, 1974) using commercially available *Bt* formulations, Dipel (*B. thuringiensis*, Serotype H3) and Bactospeine (*B. thuringiensis*, Serotype H1). Both of these formulations contain *B.thuringiensis* subsp. *kurstaki*. The conclusion of the study was that 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*.

Toxicity to birds

Avian toxicity data for 'Vectobac' technical material (*Bti AM65-52*) are limited to the results of two studies of short-term dietary toxicity (shown below) in which diets containing 'Vectobac' technical material were fed to mallard ducks and Northern bobwhite. These two studies were conducted over a 30 day period; following an initial five day dietary exposure the birds were observed for a further 25 days. The results of the studies are presented in Table 4.

Table 4 Summary of the short-term dietary toxicity of VectoBac* technical material (Bti AM65-52) to birds

Species	**Endpoint**	**Result**				**Reference**
		Dietary concentration (mg MPCA/kg)	**Daily intake (mg MPCA/kg bw/day)**	**CFU/kg bw/day**	**ITU/kg bw/day**	

Mallard duck	5-day LD_{50} NOEC	> 3077 3077	>716 716	6.2 x 10^{11}	2.03 x 10^{7}	IIIA, 8.1-01
Northern bobwhite	5-day LD_{50} NOEC	> 3077 3077	>1874 1874	6.2 x 10^{11}	2.03 x 10^{7}	IIIA, 8.1-02

* VectoBac Technical used in bioassays had a biopotency of $2x10^{11}$ CFU/g MPCA and $6.6x10^{3}$ ITU

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).

Effects on Soil Non-Target Micro-organisms

No laboratory studies with soil micro-organisms have been performed according to internationally recognised guidelines. A study has been reported (Koskella and Stotzky, 2002) using toxins from *Bti* (25 – 130 kDa) which were purified from 3 -5 day old cultures. The tests were performed using *Bacillus megaterium, B. subtilis, B. cereus, Staphylococcus faecalis* and *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 resulting from the insecticidal protein crystals from *Bti* against a variety of gram-positive bacteria were observed. It should be stressed that *S. faecalis* and *S. aureus* are not considered normal inhabitant of soil compartment

Bees

A 14-day oral toxicity study was conducted to determine the effects of 'VectoBac' technical material (*Bti* AM65-52) on adult worker honey bees (Atkins, 1990). The result of the study showed that 'VectoBac' was not a stomach poison to adult worker honey bees (*Apis mellifera* L.) feeded at dosages up to 10x 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 (Table 5).

Table 5 – Effects of Bti AM65 52 on adult worker honey bee

Organism	Test substance	Study type	mg MPCA*/bee/day	Effects	Reference
Apis mellifera (adult workers)	VectoBac* Technical	14-day oral toxicity	0.124 ($2.5x10^{7}$ CFU/bee/day; $8.2x10^{2}$ ITU/bee/day)	No observed effects	IIIA 8.5-01
Apis mellifera (adult	VectoBac WG	48 hrs	Contact toxicity: LD_{50} >100 µg ($1.8x10^{6}$	No observed effects	IIIB 10.3-01

workers)			CFU; $3x10^2$ ITU) /bee Oral toxicity : LD_{50} > 108.4 µg ($1.9x10^6$ CFU; $3.2x10^2$ ITU)/bee		

*VectoBac Technical used in bioassays had a biopotency of $2x10^{11}$ CFU/g MPCA and $6.6x10^3$ ITU/mg MPCA

Other flora and fauna

No specific studies were carried out to determine whether *Bti* AM65-52 has an impact on other flora and fauna. It is considered that sufficient data have been provided.

2.2.2.3. Exposure assessment and Risk characterisation

For the expression of metrics appropriate to microorganisms, values have been referred hereafter in EED (Expected Environmental Density) and PNED (predicted no-effect density) when dealing with viable cell counts, i.e. Colony Forming Units per Unit of weight or volume. The traditional PEC and PNEC have been kept as metrics when dealing with toxin Units per Unit of weight or volume.

Aquatic compartment (including 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.

Surface water EED/PNED calculation

To evaluate risk assessment for aquatic compartment, EED/PNED ratio has to be calculated. If EED/PNED ratio is < or equal to 1, no refinement is required, if EED/PNED is >1, a step-2 calculation (Document II B) has to be made.
For *Bti* AM65 52, the value of PNECsw is extrapolated from the EC obtained for Daphnia in reproduction test, where NOEC= 0.5 mg/L ($1x10^8$ CFU/L). Appling an AF of 10, PNEDsw is 0.05 mg/L ($1x10^7$ CFU/L). EEDsw/PNEDsw ratios for 1 and 8 treatments are indicated in Table 7.
As shown in the table, a value lower than 1 after one treatment (EED_{SW} = 5.98 x 10^6, $EED_{SW}/PNED_{SW}$ = 0.6) was calculated, but a value higher than 1 resulted after 8 applications (EED_{SW} = 3.49x 10^7, $EED_{SW}/PNED_{SW}$ = 3.5). However, as a step-2 calculation can be made assuming a distribution constant $K_{OC} = 10^3$ mL g^{-1} (a quite high value but not so unrealistic as the value of 10^6 used in EUSES in absence of adsorbtion data). The step-2 EED_{SW} following 1 application is 4.43 x 10^5 and therefore the $EED_{SW}/PNED_{SW}$ ratio is 0.004. After 8 applications EED_{SW} is 3.46 x 10^6 and the $EED_{SW}/PNED_{SW}$ ratio is 0.35.
The step-2 EED_{SW} values (i.e. considering adsorption) obtained are as follows:

Table 6 Aquatic compartment. PNEDsw derivation based on the most sensitive species

AQUATIC COMPARTMENT						
Test organism	NOEC (21 d, chronic)	AF	PNEDsw [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: $5.98x10^6$ 8 applications: $3.49x10^7$	0.6 3.5
Daphnia magna	0.5 mg/L ($1x10^8$ CFU/L)	10	0.05 ($1x10^7$ CFU/L)	2	1 application: $4.43x\ 10^5$ 8 applications: $2.59x\ 10^6$	0.04 0.26

Sediment EED/PNED calculation

An attempt to establish a quantitative risk assessment for sediments, using the methodology previously indicated for surface water, has been made and is shown in Table 7.

Table 7 EED values for sediments

EED_{sed} [CFU/g]		PNED sed
Step-1		
1 application	8 applications	
$2.39x\ 10^4$	$1.67\ x\ 10^5$	n.s.
Step-2		
$2.22\ x\ 10^4$	$1.29\ x\ 10^5$	n.s.

EED_{sed} in Step-1 has been calculated assuming the total MPCA rate as applied to the sediment. However, no PNEDsed is available.

Sewage treatment plants (STP)

No specific study on microorganisms was carried out to assess biological effects of *Bti* AM65-52 on STP microbial community.

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

Recently Mizuki *et al*. (2001) recovered at high frequency *Bt* from activated-sludge system environments in an urban sewage-digestive plant, and the highest density was $1.6\ x\ 10^3$ CFU/ml.. No antibiotic activity of the Insecticidal Crystal Proteins (ICPs) from *Bti* against a variety of gram-positive bacteria was observed.

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

Calculations of CFU amount in water following a STP treatment has been performed in a similar manner to the disposal of general industrial chemicals as laid down in the *Technical Guidance Documents (TGD) for the Risk Assessment of Existing and New Notified Industrial Chemicals* (*1996*), with some necessary modifications. Therefore, the local spore density (EED) of the biocide in surface water has been calculated ignoring elimination processes like volatilisation, degradation or sedimentation in a sewage treatment plant (STP)

Following the step-1 approach in case of one application, the concentration in STP-untreated waste water, $EED_{local,\ influent}$, $EED_{local,\ sw}$, $EED_{local,\ sed}$ are:

$EED_{local,\ influent} = 0.125$ [mg MPCA/L] $= 6.0x10^6$ [CFU/L]

$EED_{local,\ sw} = 5.99x10^5$ [CFU/L]

$EED_{local, sed} = 5.21x10^{7}$ [CFU/kg sed]

Analogously, following the same steps of calculations, in case of 8 applications the different EEDs will be:

$EED_{local, influent}$, $=3.49x10^{7}$ [CFU/L]

$EED_{local, sw} =3.5x10^{6}$ [CFU/L]

$EED_{local, sed} = 3.05x10^{8}$ [CFU/kg sed]

Following the step-2 approach, in case of one application:

$EED_{local, influent} = 4.42x10^{5}$ [CFU/L]

$EED_{local, sw} = 4.42x10^{4}$ [CFU/L]

$EED_{local, sed} = 3.84x10^{6}$ [CFU/kg sed]

and in case of 8 applications:

$EED_{local, influent} =3.46x10^{6}$ [CFU/L]

$EED_{local, sw} =3.46x10^{5}$ [CFU/L]

$EED_{local, sed} =3.00x10^{7}$ [CFU/kg sed]

Atmosphere

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, *Bti* substantial concentrations of the micro-organism will not be present in air unless aerial spray and with repeated treatments for extended time periods and consequently the micro-organism will not undergo long-range atmospheric transportation.

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

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

'VectoBac WG' when tested on adult worker honey bees (*Apis mellifera* L.) gave an acute oral 48-hour LD_{50} of > 108.4 µg ($1.9x10^6$ CFU; $3.2x10^2$ ITU) Vectobac WG/bee and an acute contact 48-hour LD_{50} of >100 µg ($1.8x10^6$ CFU; $3x10^2$ ITU) Vectobac WG/bee. 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 were 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

EED/PNED ratio at local level below 1 indicates negligible risk for the environment.
The PNED for terrestrial organisms can take into account the value of acute toxicity obtained for earthworms, corrected by an AF equal to 1000. (Table 8)

Table 8 EED/PNED calculation for earthworms

Test organism	EC (mg/kg soil)	AF	$PNED_{soil}$ [mg/L]	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: 2.39×10^4 8 applications: 1.67×10^5	0.002 0.02

$EED_{soil}/PNED_{soil}$ ratio is less than 1 for 1 and for 8 applications, so that no refinement in calculation has to be applied.
The overall conclusion on evaluation of risk assessment for terrestrial compartment is that Vectobac WG poses negligible risks to organisms of terrestrial environment.

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

Secondary poisoning concerns toxic effects in organisms at high trophic levels based on ingestion of organisms from lower trophic levels. Measured or predicted concentrations of residues in top predators are compared to no effect concentrations for the predators. The key components of the assessment of secondary poisoning are the assessment of potential bioaccumulation and potential toxicity of the substance following exposure to residues of the active substance. 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*) (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.

2.2.3. List of endpoints

In order to facilitate the work of Member States in granting or reviewing authorisations, and to apply adequately the provisions of Article 5(1) of Directive 98/8/EC and the common principles laid down in Annex VI of that Directive, the most important endpoints, as identified during the evaluation process, are listed in Appendix I.

3. DECISION

3.1. Background to the Decision

Bacillus thuringiensis subsp. *israelensis* – Strain AM65-52 has been supported and evaluated as an insecticide in the following use situations: control of mosquito and black fly larvae in water habitats and filter fly midges in sewage treatment plants.
A satisfactory methodology for the identification of Bti AM65-52 at strain level has been developed, based on genomotyping
Bti AM65-52 poses no quantifiable risk to human health in respect of its use as a microbial insecticide and it is therefore not considered necessary to set an ADI, an AOEL or a maximum allowable concentration (MAC) in drinking water. *Bti* AM65-52 is recommended for control of larvae of mosquitoes and black flies in water habitats and larvae of filter fly midges in sewage treatment plants, uses which normally do not leave residues in food or feedstuffs. It is therefore not necessary to calculate the potential exposure of consumers, or propose a Maximum Residue Level (MRL) for this micro-organism at this stage. However, should authorisation be sought for products containing *Bti* AM65-52 that could lead to residues in food or feed, it would have to be verified whether existing MRLs need to be amended. The use of *Bti* AM65-52 at the recommended concentration and rate of application is not expected to have harmful effect on human or animal health or unacceptable effect on the environment. *Bti* AM65-52 technical powder has shown sensitization in animal models at concentration greater than 0.5% w/v, which are unlikely to be reached following biocide use.

3.2. Decision regarding Inclusion in Annex I

The organism *Bacillus thuringiensis* subsp. *israelensis*, Serotype H-14, Strain AM65-52 shall be included in Annex I to Directive 98/8/EC as an active substance for use in product-type 18 (Insecticide), subject to the following specific provisions:

- When assessing the application for authorization of a product in accordance with Article 5 and Annex VI, Member States shall assess, where relevant for the particular product, those uses or exposure scenarios and those risks to human populations and to environmental

compartments that have not been representatively addressed in the Union level risk assessment.

- Products authorized for professional use shall be used with appropriate personal protective equipment, unless it can be demonstrated in the application for product authorization that risks to professional users can be reduced to an acceptable level by others means.
- For products containing *Bacillus thuringiensis* subsp. *israelensis* Serotype H14, Strain AM65-52 that may lead to residues in food or feed, Member States shall verify the need to set new or amended existing maximum residue levels (MRLs) according to Regulation (EC) No 470/2009 or Regulation (EC) No 396/2005, and take any appropriate risk mitigation measures ensuring that the applicable MRLs are not exceeded.

3.3. Elements to be taken into account by Member States when authorising products

The following elements are to be taken into account by MSs when authorizing product**:**

- Bti AM65-52 may cause a sensitization 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.

3.4. 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.

3.5. Updating this Assessment Report

This assessment report may need to be updated periodically in order to take account of scientific developments and results from the examination of any of the information referred to in Articles 7, 10.4 and 14 of Directive 98/8/EC. Such adaptations will be examined and finalised in connection with any amendment of the conditions for the inclusion of *Bacillus thuringiensis* subsp. *israelensis* strain AM65-52 in Annex I to the Directive.

Appendix I: List of endpoints

Chapter 1: Identity, Biological Properties, Classification and Labelling

Active substance	*Bacillus thuringiensis* subsp. *israelensis* Serotype H-14 Strain AM65-52
Function (*e.g.* fungicide)	Biological larvicide

Identity

Common name	*Bacillus thuringiensis* subsp. *israelensis* Serotype H-14 Strain AM65-52 (abbreviated to *Bti* AM65-52).	
Taxonomic name	Species:	*thuringiensis*
	Subspecies:	*israelensis*
	Serotype:	H-14
	Strain:	AM65-52
	Genus:	*Bacillus*
	Family:	Bacillaceae
Collection and culture reference number	SD-1276 American Type Culture Collection.	
Other substance No.	None	
Minimum purity of the active substance as manufactured (g/kg or g/L)	The technical grade of *Bti* AM65-52 is a fermentation slurry that contains the bacillus, spores and insecticidal toxins and solid residues from the fermentation. Fermentation residues will include the original components of the fermentation medium, plus metabolic and excretion products from the growing bacteria. The fermentation slurry contains nominally 14% (4.8×10^{10} cfu/g) *Bti* AM65-52, with high and low limits of 20% (6.8×10^{10} cfu/g) and 8% (2.7×10^{10} cfu/g) respectively.	
Identity of relevant impurities and additives (substances of concern) in the active substance as manufactured (g/kg)	There are no substances of concern in the active substance as manufactured.	

Source and biological properties

Natural occurrence and distribution	*Bacillus thuringiensis* subsp *israelensis* is a common naturally occurring micro-organism with worldwide distribution. The

	species has been detected both in soil and on insects and plants and will be indigenous to intended areas of application.
Isolation methods	The origin of the strain used for the production of 'VectoBac' products is confidential to Valent BioSciences and information relating to this is contained in the confidential attachment under Point IIIA 2.1.2.
Culture methods	Culture methods are confidential to Valent BioSciences and information relating to this is contained in the confidential attachment.
Production methods	The production method is confidential to Valent BioSciences and information relating to this is contained in the confidential attachment.
Composition of the micro-organism	The composition of the micro organism is confidential to Valent BioSciences and information relating to this is contained in the confidential attachment.
Methods to preserve seed stock	Methods to preserve the seed stock are confidential to Valent BioSciences and are contained in the confidential attachment.
Relationship to existing pathogens	*Bacillus anthracis* and *Bacillus cereus* are bacterial species related to *Bacillus thuringiensis*. *Bacillus anthracis* is known to cause anthrax in humans and animals, whilst *Bacillus cereus* is known to cause gastro-intestinal disorders in humans. *Bti* AM65-52 can be clearly distinguished from these other *Bacillus* species and strains. There are no other active metabolites and degradation products that are known to contribute to the toxicity of *Bti* AM65-52. The presence of beta-exotoxins and enterotoxins which may be produced by other *Bacillus thuringiensis* subspecies is monitored and controlled during production and do not occur in *Bti* AM65-52.
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
Transmissibility, infective dose and mode of action and information on the nature, identity and stability of toxins	Bti does not act on target organisms by transmission or infection.
Infectivity and stability in use	*Bacillus thuringiensis* species are not infective within populations of the target organism and do not multiply substantially in the cadaver. Re-infection in the field after

	application is not expected to occur. *Bacillus thuringiensis* species can be considered a natural part of the microflora in the environment
Genetic stability	Gene transfer to and from *Bacillus thuringiensis* bacteria are a natural events in the environment, however gene transfer will only take place in the presence of metabolically active bacteria. *Bacillus thuringiensis* is present primarily in its spore form in the environment and therefore opportunity for gene transfer may be regarded as negligible. Furthermore, plasmid transfer has been shown to be difficult in non-sterile soils and therefore natural gene transfer may not be common.
Resistance or sensitivity to antibiotics	*Bti* AM65-52 is susceptible to various commercial antibiotics, but is resistant to others. This information is available in the confidential attachment.

Classification and labeling

with regard to biological properties	Not classified
with regard to toxicological data	Not classified
with regard to fate and behaviour data	Not classified
with regard to ecotoxicological data	Not classified

Chapter 2: Methods of Analysis

Analytical methods for the active substance

Technical active substance	Characterisation of strain or serotype within *Bacillus thuringiensis* species is commonly performed using classical techniques such as; crystal morphology, biochemical reactions and bioassays. Recent advances in molecular biology have allowed the development of specific DNA based methods capable of distinguishing individual strains and isolates, including Strain AM65-52. The specific methods used are confidential to Valent BioSciences and are included with all other confidential information in the confidential attachment.
Identity and impurities in the seed stock	The maintenance of the *Bti* AM65-52 seed stock is confidential to Valent BioSciences and information relating to this is contained in the confidential attachment.

Microbiological purity	Each lot of fermentation solids and soluble concentrate is tested for mammalian safety using a mouse safety test prior to addition of other formulation ingredients. Sterility testing procedures are used for microbial purity and sterility monitoring of the seed stock and fermentors. Details of these methods are confidential to Valent BioSciences and are presented in the confidential attachment.
Presence of pathogens	Enterotoxins can be detected using commercially available immunoassay kits. The absence of Type I and Type II beta-exotoxin is determined by HPLC or fly bioassay. Periodic monitoring of production batches is performed to provide assurance that beta-exotoxins are not produced.

Analytical methods for residues

Food	Contamination of food items is not anticipated following use and MRL's in food are not established for *Bti* AM65-52. Monitoring methods in food are therefore not relevant.
Feed	Contamination of feed items is not anticipated following use and MRL's in feed are not established for *Bti* AM65-52. Monitoring methods in feed are therefore not relevant.
Animal tissues	Contamination of animal tissues is not anticipated following use and MRL's in animal tissues are not established for *Bti* AM65-52. Monitoring methods in animal tissues are therefore not relevant.
Soil	The use pattern of the product means there is negligible potential for *Bti* AM65-52 vegetative cells, spores or parasporal crystals to enter soil at concentrations significantly greater than those present naturally. Methods in soil are therefore not considered relevant.
Water	*Bti* AM65-52 is rapidly inactivated in water by sorption onto particulate matter and is harmless to non-target species and humans. Furthermore, *Bti* AM65-52 is not used on clean drinking water. Methods for *Bti* AM65-52 in water are therefore not considered necessary.
Air	The use pattern of the product means there is negligible potential for *Bti* AM65-52 vegetative cells, spores or parasporal crystals to enter the air at concentrations significantly greater than those present naturally. Methods in air are therefore not considered relevant.

Chapter 3: Impact on Human Health

Basic information	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.
Sensitisation:	Animal models, topical application in a Buehler design study with the active ingredient and an M&K design for the product, 'VectoBac' WG, indicated a mild sensitising potential for the active material and no sensitising properties for the product. The potential identified in the animal models has not been realised in the exposed human population.
Acute oral toxicity, pathogenicity and infectivity:	The oral LD_{50} of *Bacillus thuringiensis* in rats was > 5000 mg/kg. Oral administration of 10^8 CFU/animal had no adverse effects on rats and was neither infective nor pathogenic. The oral LD_{50} of 'VectoBac' WG, was determined to be greater than 5000 mg/kg bw in rats
Acute inhalation toxicity, pathogenicity and infectivity:	Intratracheal instillation of 10^8 CFU technical material to rats resulted in no mortality, pathogenicity or infectivity. Spores persisted in some tissues with clearance estimated to take up to 100 days. In a second study the dose was reduced to 10^7 CFU, clearance from the lungs was largely completed by Day 22 in controls but values of 10^6 CFU remained in treated animals No signs of infectious disease were apparent. Rats exposed to the undiluted bacterial spores, presented as an aerosol for 4 hours, at the maximum attainable chamber concentration of 2.84 mg/L air, showed no clinical signs after Day 1 and there were no deaths. A concentration of 2.84 mg/L was therefore considered to be a NOAEL and the acute LC_{50} exceeded 2.84 mg/L. The acute inhalation LC_{50} of the product, 'VectoBac' WG was greater than the maximum achievable dose level of 0.014 mg/L when administered undiluted as an aerosol to albino rats

Intraperitoneal/subcutaneous single dose:	Acute intravenous administration to rats of approximately 10^7 CFU resulted in no treatment related toxicity and no evidence of pathogenicity. Intraperitoneal injection of 10^6, 10^7 or 10^8 CFU/g to mice resulted in no signs of toxicity or pathogenicity. None of the studies with *Bti* (Strain AM65-52) showed signs of infectivity or pathogenicity by routes of maximum challenge. Effects were only observed in some strains at very high doses (10^8 CFU) injected directly into the brain.
In vitro genotoxicity:	Tests for genotoxicity are required if the micro-organism produces exotoxins (defined in point 2.8 as "*metabolites (especially toxins) with unacceptable effects on human health and/or environment during or after application*". The toxicity, infectivity and pathogenicity investigations completed for *Bti* indicate toxins with such adverse effects on human health are not present. For genotoxicity one would expect covalent binding of a genotoxic compound to the target-tissue DNA. There has been no evidence throughout the years that *Bti* has been used in vector control programs that it produces any toxin capable of DNA binding. In the waiver request submitted it is argued that standard mutagenicity and genotoxicity assays are not considered appropriate for many living micro-organisms nor does the risk they pose often warrant such testing.
Cell culture study:	Cell culture studies are required for viruses and viroids or specific bacteria and protozoa with intracellular replication. This is not applicable to *Bacillus thuringiensis* which does not replicate in warm-blooded organisms.
Information on short-term toxicity and pathogenicity:	No evidence for 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 1.84×10^6 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*.
Dermal toxicity:	The median lethal dermal dose level (LD_{50}) of *Bacillus thuringiensis* in rabbits was found to be greater than 5000 mg/kg. The dermal LD_{50} of 'VectoBac' WG was determined to be greater than 5000 mg/kg bw in rats.
Specific-toxicity, pathogenicity and infectivity:	In a range of toxicological studies, completed using serotype H-14 of *B. thuringiensis*, experimental infection of mice, rats, guinea pigs and rabbits was attempted by various routes. Doses in the range of 10^7 to 10^8 CFU resulted in no adverse

	toxic effects following acute or repeated exposure. There were no indications of anaphylaxis in guinea pigs and repeated passage through mice showed no signs of virulence. It was conclude that serotype H-14 of *B. thuringiensis* was well tolerated by the test species used, showed no propensity to multiply within the host and was rapidly eliminated without causing adverse effects. Serotype H-14 was confirmed to be innocuous.
Genotoxicity – in vivo studies in germ cells:	*In vivo* testing of *Bacillus thuringiensis* subsp. *israelenis* is not indicated.
Exposure (operator, workers, bystanders, consumer):	Since the recommended testing regimen is largely limited to acute exposure, based on 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. No monitoring data are submitted from studies investigating operator or worker exposure. The active material 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

Chapter 4: Fate and Behaviour in the Environment

Spread, mobility, multiplication and persistence in air, soil and water	Degradation of *Bti*-vegetative cells and insecticidal toxins in soil (DT_{50} = 5.2 days) and poor germination of *Bti* 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 discernable 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.

A two-steps approach has been used for the calculation of both EEDs (CFU) and PECs (ITU).

<u>First-step assumptions and calculations</u>

Direct applications to soil of 1 kg/ha VectoBac ($4.8x10^{10}$ CFU/g MPCA and $3x10^{6}$ ITU/g MPCP) containing 37.4 % *Bti* AM65-52. First order degradation rate, no adsorption, no plant interception..

Following 1 application:

$EED_{S,time=0} = 2.4x10^{4}$ CFU/g

$PEC_{S,time=0} = 4$ ITU/g

Following 8 applications with intervals of 7 days:

$EED_{S,time=0} = 1.7x10^{5}$ CFU/g

$PEC_{S,time=0} = 6.6$ ITU/g

Direct applications to a water body, having a depth of 30 cm and an area of 1 ha, of 1 kg/ha VectoBac ($4.8x10^{10}$ CFU/g MPCA and $3x10^{6}$ ITU/g MPCP) containing 37.4% *Bti* AM65-52. First order degradation rate, no adsorption.

Following 1 application:

$EED_{SW,time=0} = 6x10^{6}$ CFU/L

$PEC_{SW,time=0} = 1x10^{3}$ ITU/L

Following 8 applications with intervals of 7 days:

$EED_{SW,time=0} = 3.5x10^{7}$ CFU/L

$PEC_{SW,time=0} = 3.2x10^{3}$ ITU/L

<u>Second-step assumptions and calculations</u>

Direct applications to a water body, having a depth of

30 cm and an area of 1 ha, of 1 kg/ha VectoBac ($4.8x10^{10}$ CFU/g MPCA and $3x10^6$ ITU/g MPCP) containing 37.4% *Bti* AM65-52. First order degradation rate, adsorption (assumed Koc=1000) on a sediment having a bulk density of 1.5 g/cm^3 and a thickness of 5 cm.

Following 1 application:

$EED_{SW,time=0}$= $4.4x10^5$ CFU/L

$PEC_{SW,time=0}$=74 ITU/L

$EED_{Sed,time=0}$= $2.2x10^4$ CFU/g

$PEC_{Sed,time=0}$=3.7 ITU/g

Following 8 applications with intervals of 7 days:

$EED_{SW,time=0}$= $2.6x10^6$ CFU/L

$PEC_{SW,time=0}$=$2.4x10^2$ ITU/L

$EED_{Sed,time=0}$= $1.3x10^5$ CFU/g

$PEC_{Sed,time=0}$=12 ITU/g

Bti-is not infectious and has a limited survival in the environment resulting in a limited spread of the organism. Vegetative cells and insecticidal toxins of *Bti* have a limited survival time in the environment and *Bti* spores do not germinate readily, making it unlikely that *Bti* AM65-52 will multiply and colonise areas of intended use. *Bti* vegetative cell and insecticidal toxins are fairly persistent, not mobile in soil and not persistent in water. Airborne concentrations of *Bti* AM65-52 are expected to be negligible following application to water bodies and sewage. Aerial concentration following aerial treatments are expected to be fairly persistent.

Chapter 5: Effects on Non-target Species

Effects on birds		
Species	**Time-scale**	**Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects)**
Mallard duck	Short-term	No apparent pathogenicity, toxicity or effect upon survival of young mallards when administered by oral gavage at 3077 mg/kg bw per day (equivalent to a daily dose of 716 mg/kg bw/day), an equivalent of approximately 6.2×10^{11} CFU/kg of body weight per day ($2.07x10^7$ ITU/kg bw/day) for five days followed by 25 days of observation.

		The LC_{50} was >3077 mg/kg per day (6.2 x 10^{11} CFU/kg bw/ day; 2.07x10^{7} ITU/kg bw/day) (equivalent to a daily dose of 716 mg/kg bw/day).
Northern bobwhite	Short-term	No apparent pathogenicity, toxicity or effect upon survival of young Northern bobwhite when administered by oral gavage at 3077 mg/kg per day for five days (equivalent to a daily dose of 1874 mg/kg bw/day), an equivalent of approximately 6.2 x 10^{11} CFU/kg of body weight per day (2.07x10^{7} ITU/kg bw/day) followed by a further 25 day observation period. The LC_{50} was >3077 mg/kg per day (6.2 x 10^{11} CFU/kg bw/ day; 2.07x10^{7} ITU/kg bw/day) (equivalent to a daily dose of 1874 mg/kg bw/day).

Effects on aquatic organisms			
Group	**Test substance**	**Time-scale**	**Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects)**
Laboratory tests – fish species			
Oncorhynchus mykiss	*Bti*	Acute 96-hours	96h LC_{50} >370 mg MPCA/L
Lepomis macrochirus	*Bti*	Acute 96-hours	96h LC_{50} >600 mg MPCA./L
Oncorhynchus mykiss	'Vectobac' technical	Chronic 32-day	NOEC - 1.1x 10^{10} CFU/L aqueous exposure (3.7x10^{5} ITU/L), 1.72 x 10^{10} CFU/g (5.7x10^{5} ITU/g) dietary exposure. No adverse effects to the fish based on survival, infectivity or pathogenicity were observed during the 32-day exposure period. Fish growth in the VectoBac treatment was significantly lower than in the control, an effect which may be due in part to the high turbidity and suspended solids encountered in the test solution..
Lepomis macrochirus	'Vectobac' technical	Chronic 30-day	NOEC - 1.2x 10^{10} CFU/L (4x10^{5} ITU/L) aqueous exposure, 1.31 x 10^{10} CFU/g (4.4x10^{5} ITU/g) dietary exposure. No adverse effects to the fish based on survival, growth, infectivity or pathogenicity observed during the 30-day exposure period.
Cyprinodon variegatus	'Vectobac' technical	Chronic 30-day	NOEC - 1.3x 10^{10} CFU/L (4.3x10^{5} ITU/L) aqueous exposure, 2.1 x 10^{10} CFU/g (7x10^{5} ITU/g) dietary exposure. No adverse effects to the fish based on survival, growth, infectivity or pathogenicity observed during the 30-day exposure period.

Laboratory tests – invertebrate species			
Daphnia magna	'Vectobac' technical	Chronic 10-day	10-day LC_{50} - >50 mg MPCA/L ($3.6x10^9$ CFU/L)
Daphnia magna	'Vectobac' technical	Chronic 21-day	NOEC - 0.5 mg MPCA/L ($1 x 10^8$ CFU/L; $3.3x10^3$ ITU/L)
Grass shrimp (*Palaemonetes vulgaris)*	'Vectobac' technical	Chronic 31-day	NOEC - $2.0 x 10^{10}$ CFU/g ($6.6x10^5$ ITU/g) dietary concentration No adverse effects to shrimp based on survival, growth, no signs of infectivity, tumours, necrosis, abnormal behaviour or pathogenicity observed during the 31-day exposure period.
Mayfly nymphs (*Hexagenia* sp)	'Vectobac' technical	Chronic 18-day	NOEC - $2.0 x 10^{10}$ CFU/L ($6.6x10^5$ ITU/g) aqueous concentration No adverse effects to mayfly nymphs based on survival, growth, no signs of infectivity, tumours, necrosis, abnormal behaviour or pathogenicity observed during the 18-day exposure period.
Amphiascus minutus	'Vectobac' technical	Chronic 10-day	10-day LC_{50} - >50 mg MPCA/ kg sediments (>$1x10^{10}$ CFU/kg sediments; >$3.3x10^5$ ITU/kg sediments) NOEC - >50 mg a.s./ kg sediments (>$1x10^{10}$ CFU/kg sediments; >$3.3x10^5$ ITU/kg sediments)
Field study - invertebrates			
Natural assemblage of aquatic invertebrate fauna	'VectoBac'-G, (*Bti* spores and crystals associated with corn cobs)	Chronic	Repeated application of VectoBac-G (*Bacillus thuringiensis israelensis*, AM65-52) did not affect density of total insects, Diptera, non-dipterans, *Chironomidae*, predators, and non-insect benthic invertebrates. Biomass comparisons between treatments showed a very similar pattern to the density results.
Effects on algae (growth, growth rate, capacity to recover)			
A study with *Bti* toxins showed that pure *Euglena* spp, *Chlamydomonas* sp., *Oedogonium* sp, mixed algal cultures and a cyanobacterium (*Oscillatoria* sp) were not inhibitory in dilution tests. 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 to enable the release of the active protein delta endotoxins.			
Effects on plants other than algae			
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 delta endotoxins.			

*test performed with a MPCA containing $7.2x10^{10}$ CFU/g. No ITU content was indicated

Effects on bees		
Species	**Route**	**Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects)**
Honey bee	Oral	A 14-day oral toxicity study was conducted to determine the effects of 'VectoBac' technical material on adult worker honey bees. The result of the study showed that 'VectoBac' (*Bacillus thuringiensis* var. *israelensis)* was not a stomach poison to adult worker honey bees (*Apis mellifera* L.) at dosages ranging from 0.5 to 10 times (2400 g/acre correspondent to 5931 g/ha; $1.2x10^{15}$ CFU/ha; $4x10^{10}$ ITU) the field rate,. 'VectoBac WG' when tested on adult worker honey bees (*Apis mellifera* L.) gave an acute oral 48-hour LD_{50} of > 108.4 µg ($1.9x10^{6}$ CFU; $3.2x10^{2}$ ITU) Vectobac WG/bee and an acute contact 48-hour LD_{50} of >100 µg ($1.8x10^{6}$ CFU; $3x10^{2}$ ITU) Vectobac WG/bee. On the basis of these results 'VectoBac' can be classified as essentially not-toxic to honey bees.

Effects on other arthropods species
Information is available on the May fly, see previous page under Laboratory tests – invertebrate species. Information can also be found in the Field Studies quoted above.

Effects on non-arthropod invertebrates	
Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects)	*Eisenia fetida* 30-day LC_{50} value of >1000 mg/kg dry weight soil. Under the conditions of the study, 'Vectobac' technical powder (*Bacillus thuringiensis* subsp. *israelensis*) was neither toxic nor pathogenic to earthworms. In a field trial the application of two commercial formulations of *Bacillus thuringiensis* (Dipel and Bactospeine) at 100 times the recommended application rates had no effect on earthworm density nine weeks after application.
Further information:	No further information

Effects on non-target soil micro-organisms
A study on the effects on non-target soil micro-organisms is reported under data point IIM 8.4. 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 (*Bacillus megaterium*, *B. subtilis*, *B. cereus*, *Staphylococcus faecalis*, or *Staphylococcus aureus*) regardless of whether the cultures were incubated under starvation or non-starvation conditions. No antibiotic activity of the Insecticide Crystal Proteins (ICPs) from *Bti* against a variety of gram-positive bacteria were observed.

Measures necessary to protect man, animals and the environment

1 Recommended methods and precautions concerning handling, use, storage, transport or fire	
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.
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.
1.3 Methods and precautions concerning storage	Store under cool, dry and well-ventilated conditions. Keep away from food, drink and animal feed stuffs.
1.4 Methods and precautions concerning transport	There are no restrictions for *Bti* AM65-52 or 'VectoBac' WG concerning transport by land, sea or air.
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 Specific treatment in case of an accident, e.g. first-aid measures, antidotes, medical treatment if available; emergency measures to protect the environment	
2.1 Specific treatment in case of an accident, e.g. first-aid measures, antidotes, medical treatment if available	*Bti* AM65-52 it has to be considered as non toxic by acute exposure and first aid measures and a specific therapeutic regimen cannot 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, seek medical attention. Provide symptomatic/supportive care as necessary. TREATMENT: Supportive therapy, antibiotics may be used. WARNING: Cannot 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.
2.2 Emergency measures to protect the environment	*Bti* AM65-52 does not pose unacceptable risks to non-target species and specific measures to protect the environment are not necessary.
3 Procedures, if any, for cleaning application equipment	Application equipment should be cleaned using normal cleaning procedures.
4 Identity of relevant combustion products in cases of fire	*Bti* AM65-52 is not flammable or oxidising. None of the components in 'VectoBac' WG contain halogens. In the event of a fire 'VectoBac' WG 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.
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* AM65-52 and any associated contaminated packaging should be disposed in accordance with governmental or local authority regulations. If further advice is required contact the manufacturer. Depending on situations and if a centralized collection and recycling infrastructure is in place for pesticide containers, packaging materials are often made from recyclable materials. Properly cleaned plastic containers can be recycled. Recycling is the best management option for containers.
6 Possibility of destruction or decontamination following release onto:	
6.1 Air	Bti AM65-52 does not pose unacceptable risks in air. Therefore no special requirements are needed to render the micro-

	organism harmless in air.
6.2 Water, including drinking water	*Bti* AM65-52 cannot be used on treated water for drinking or on non chlorinated waters for recreational purposes.
6.3 Soil	*Bti* AM65-52 does not pose unacceptable risks to non-target species and showed no acute toxicity on humans and therefore no special requirements are needed to render the micro-organism harmless in soil.
7 Observations on undesirable or unintended side-effects, e.g. on beneficial and other non-target organisms	*Bti* AM65-52 does not pose unacceptable risks to non-target species and humans and no undesirable or unintended side effects are anticipated.
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* AM65-52 does not contain any repellents or poison control measures in the preparation.

APPENDIX II: LIST OF INTENDED USES

Field of use/ Product type	**Application type**	**Number and timing of application**	**Waiting periods**	**Information on recommended variations of the application rate in different locations**	**Remarks**
Control of mosquito and black fly larvae in water habitats and filter fly midges in sewage treatment plants. Product Type 18	Ground application: tractor-mounted or hand-held sprayer.	*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. The maximum number of applications is up to 8.	None	250 to 1000 g product/ha (4.5 x 10^{12} to 1.8 x 10^{13} CFU/ha; 7.5 x 10^{8} to 3 x 10^{9} ITU/ha). Product is diluted in water and applied as a spray at 50 to 1000 L water/ha (ground application: tractor-mounted or hand-held sprayer) or 2.5 to 100 L water/ha (aerial application: fixed wing or helicopter). Therefore, spray concentration = 0.0094 – 0.748 kg a.s./hL (ground) 0.094 – 15 kg a.s./hL (aerial)	data were provided and accepted in support of these intended uses.]

APPENDIX III: LIST OF STUDIES

Data protection is claimed by the applicant in accordance with Article 12.1(c) (i) and (ii) of Council Directive 98/8/EC for all study reports marked "Y" in the "Data Protection Claimed" column of the table below. For studies marked Yes(i) data protection is claimed under Article 12.1(c) (i), for studies marked Yes(ii) data protection is claimed under Article 12.1(c) (ii). These claims are based on information from the applicant. It is assumed that the relevant studies are not already protected in any other Member State of the European Union under existing national rules relating to biocidal products. It was however not possible to confirm the accuracy of this information.

DOC I AND DOC II-A, II-B, II-C

Section No / Reference No	**Author(s)**	**Year**	**Title. Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published**	**Data Protection Claimed (Yes/No)**	**Owner**
(Doc. I – Section 2.1.2)	Lucchini S., Thompson A., Hinton J.C.D.	2001	Microarrays for microbiologists. *Microbiology* **147**, 1403-1414.	No	No
(Doc. I – Section 2.1.2)	Broderisk, N.A., Raffa, K.F.and Handelsman, J.	2006	Midgut bacteria required for *Bacillus thuringiensis* insecticidal activity. *PNAS* **103**, 15196-15199	No	No
(Doc. I – Section 2.1.2 **Doc.IIA – Section 2.3)**	Bravo, A., Gill, S.S. and Soberon, M.	2007	Mode of action of *Bacillus thruringiensis* Cry and Cyt toxins and their potential for insect control. *Toxicology*, **49**, 423-435	No	No

Section No / Reference No	**Author(s)**	**Year**	**Title. Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published**	**Data Protection Claimed (Yes/No)**	**Owner**
(Doc. I – Section 2.1.2)	Hernandez-Soto, A.; Rincon-Castro, M. C. del; Espinoza, A. M.; Ibarra, J. E.; del Rincon-Castro, M. C.	2009	Parasporal body formation via overexpression of the Cry10Aa toxin of *Bacillus thuringiensis* subsp. *israelensis*, and Cry10Aa-Cyt1Aa synergism. *Applied and Environmental Microbiology*, **75**, (14) 4661-4667	No	No
(Doc. I – Section 2.1.2)	Barker, M., Thakker, B. and Priest, F.G	2005	Multilocus sequence typing reveals that *Bacillus cereus* strains isolated from clinical infections have distinct phylogenetic origins. *FEMS Microbiol. Lett.* **245**, 179-184.	No	No
(Doc. I – Section 2.1.2)	Carazzo; B, Negrisolo, E., Carraro, L., Alberghini, L. Patarnello, T and Giaccone, V.	2008	Multiple-Locus sequence typing and analysis of toxin genes in *Bacillus cereus* food-borne isolates. *Appl. Environ. Microbiol*. **74**, 850-860.	No	No
(Doc. I – Section 2.1.2)	Hoffmaster; A.R., Novak, R.T., Marston, C.K., Gee, J.E. Helsel, L., Pruckler, J.M. and Wilkins, P.P	2008	Genetic diversity of clinical isolates of *Bacillus cereus* using multilocus sequence typing. *Microbiology* **8**, 191	No	No

Section No / Reference No	**Author(s)**	**Year**	**Title. Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published**	**Data Protection Claimed (Yes/No)**	**Owner**
(Doc. I – Section 2.4.1)	P.A.W. Martin	1991	Dynamics of *Bacillus thuringiensis* turnover in soil, p. 315. Abst: The General Meeting of the American Society for Microbiology, 1991. Am. Soc. Microbiol.	No	No
(Doc. I – Section 2.4.1)	C. Vettori, D. Paffetti, D. Safena, G. Stotzky and R. Giannini	2003	Persistence of toxins and cells of *Bacillus thuringiensis* subsp. *kurstaki* introduced in sprays to Sardinia soils. *Soil Biol. Biochem*. **35**, 1635-1642.	No	No
(Doc. I – Section 2.4.1)	S. DeRespinis, A. Demarta, N. Patocchi, P. Luthy, R. Peduzzi and M. Tonella	2006	Molecular identification of *Bacillus thuringiensis* var. *israelensis* to trace its fate after application as a biological insecticide in wetland ecosystems. *Lett. Appl. Microbiol*. **43**, 495-601.	No	No
(Doc. I – Section 2.4.1)	A.W. West	1984	Fate of the insecticidal, proteinaceous parasporal crystal of *Bacillus thuringiensis* in soil. *Soil Biol. Biochem*. **16**, 357-360.	No	No
(Doc. I – Section 2.4.1	Akiba Y.	1986	Microbial Ecology of *Bacillus thuringiensis* VI. Germination of *Bacillus thuringiensis* spores in the soil. *Appl. Ent. Zool.* **21**, 76-80.	No	No

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(Doc. I – Section 2.4.1)	Martin P.A.W. Travers R.S.	1989	Worldwide abundance and distribution of *Bacillus thuringiensis* isolates. *Appl. Environ. Microbiol*. **55**, 2437-2442.	No	No
(Doc. I – Section 2.4.1)	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. Environmental Project No. 316. Danish Environmental Protection Agency, Denmark.	No	No
(Doc. I – Section 2.4.1)	Pedersen J.C., Damgaard P.H., Ellemberg J. Hansen B.M.	1995	Dispersal of *Bacillus thuringiensis* var. *kurstaki* in an experimental cabbage field. *Can. J. Microbiol*. **41**, 118-125.	No	No
(Doc. I – Section 2.4.1)	West A.W., Burges H.D., Dixon T.J., Wyborn C.H.	1985	Survival of *Bacillus thuringiensis* and *Bacillus cereus* spore inocula in soil: effects of pH, moisture, nutrient availability and indigenous microorganisms. *Soil Biol. Biochem.* **17**,657-665.	No	No
(Doc. I – Section 2.4.1)	Pruett C.J.H., Burges H.D., Wyborn C.H.	1980	Effect of exposure to soil on potency and spore viability of *Bacillus thuringiensis*. *J. Invert. Pathol*. **35**,168-174.	No	No
(Doc. I – Section 2.4.1)	Tapp H. Totzky SG.	1995	Insecticidal activity of the toxins from *Bacillus thuringiensis* subspecies *kurstaky* and *tenebrionis* adsorbed and bound on pure and soil clays. Appl. Environ. Microbiol. **61**, 1786-1790	No	No

Section No / Reference No	**Author(s)**	**Year**	**Title. Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published**	**Data Protection Claimed (Yes/No)**	**Owner**
(Doc. I – Section 2.4.1)	Crecchio C. Stotzky G.	1998	Insecticidal activity and biodegradation of the toxin from *Bacillus thuringiensis* subsp. *kurstaki* bound to humic acids from soil. *Soil Biol. Biochem*. **30**,463-470.	No	No
(Doc. I – Section 2.4.1)	Crecchio C., Stotzky G.	2001	Biodegradation and insecticidal activity of the toxin from *Bacillus thuringiensis* subsp. *Kurstaki* bound on complexes of montmorillinite-humic acids Al hydoxypolymers. *Soil. Biol. Biochem*. **33**, 573-581.	No	No
(Doc. I – Section 2.4.1)	Glare T.R., O'Callaghan M	2000	Bacillus thuringiensis: Biology, Ecology and Safety. John Wiley, N.Y.	No	No
(Doc. I – Section 2.4.1)	Menon A.S., De Mestral J.	1985	Survivalof *Bacillus thutingiensis* var. *kurstaki* in water. *Water air soil Pollut.* **25**, 265-274.	No	No
(Doc. I – Section 2.4.1)	Grieco V.M., Spencer K.D.	1978	Inactivation of *Bacillus thuringiensis* spores by ultraviolet and visible light. *Appl. Environ. Microbiol*. **35**, 906-910.	No	No
(Doc. I – Section 2.4.1)	Myasnik M., Manasherob R., Ben-Dov E., Zaritsky A., Margalith Y., Barak Z.	2001	Comparative sensibility To UV-B radiation of two *Bacillus thuringiensis* subspecies and other *Bacillus* sp. *Curr. Microbiol*. **43**, 140-143.	No	No

Section No / Reference No	**Author(s)**	**Year**	**Title.** **Source (where different from company)** **Company, Report No.** **GLP (where relevant) / (Un)Published**	**Data Protection Claimed (Yes/No)**	**Owner**
(Doc. I – Section 2.4.1)	Pusztai M., Fast P., Gringorten L., Kaplan H., Lessard T., Carey P.R.	1991	The mechanism of sunlight – mediated inactivation of *Bacillus thuringiensis* crystals. *Biochem. J*. **273**, 43-47.	No	No
(Doc. I – Section 2.4.1)	Teschke K., Chow Y., Bartlett K., Ross A., Van Netten C.	2001	Spatial and temporal distribution of airborne *Bacillus thuringiensis* var *kurstaki* during an aerial spry program for gypsi moth eradication. *Environ. Health Perspective* **109**, 47-54.	No	No
(Doc. I – Section 2.4.3)	Hershey A.E., Lima A.R., Niemi G.J. and Regal R.R.,	**1998**	Effects of B*acillus thuringiensis israelensis* (bti) and methoprene on nontarget macroinvertebrates in Minnesota wetlands. *Ecological Applications:* Vol. 8, No. 1, pp. 41-60.	No	No
(Doc. I – Section 2.4.3)	Pont, D., E. Franquet, and J. N. Tourenq	1999	Impact of different *Bacillus thuringiensis* variety *israelensis* treatments on a chironomid (Diptera: Chironomidae) community in a temporary marsh. J Econ Entomol 92:266–272.	No	No

Section No / Reference No	**Author(s)**	**Year**	**Title.** **Source (where different from company)** **Company, Report No.** **GLP (where relevant) / (Un)Published**	**Data Protection Claimed (Yes/No)**	**Owner**
(Doc. I – Section 2.4.3)	Tilquin M. Paris M., Reynaud S., Despres L., Ravanel P., Geremia R.A., and Gury J	2008	Long Lasting Persistence of *Bacillus thuringiensis* Subsp. *israelensis* (*Bti*) in Mosquito Natural Habitats_ PLoS ONE. 2008; 3(10): e3432. doi: 10.1371/journal.pone.0003432.	No	No
(Doc. I – Section 2.4.3)	Balcer, M. D., K. I. Schmude, J. Snitgen, and A. R. Lima.	1999	Long-term effects of the mosquito control agents Bti (*Bacillus thuringiensis israelensis*) and methoprene on non-target macroinvertebrates in wetlands in Wright County, Minnesota (1997–1998). Report to Metropolitan Mosquito Control District,. St. Paul, Minnesota. 76. plus appendices.	No	No
(Doc. I – Section 2.4.3)	Schmude, K. I., Balcer, M. D., & Lima, A. R.	1997	Effects of the mosquito control agents *Bti* (*Bacillus thuringiensis israelensis*) and methoprene on non-target macroinvertebrates in wetlands in Wright County, Minnesota (1997). Report to Metropolitan Mosquito Control District, St. Paul, Minnesota. 28pp. plus appendices.	No	No

Section No / Reference No	Author(s)	Year	Title. Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published	Data Protection Claimed (Yes/No)	Owner
(Doc. I – Section 2.4.3)	Becker N	2005	Biological control of mosquitoes: management of the Upper Rhine mosquito population as a model program. In: An ecological and societal approach to biological control - Eilenberg J., Hokkanen Heikki M. T. Eds., Chapt.11: Pag.227-245	No	No
(Doc. I – Section 2.4.3)	Lacey L.A., Merritt R.W.,	2003	The safety of bacterial microbial agents used for black fly and mosquito control in aquatic environments. In: Environment impact of microbial insecticides. Need and methods for risk assessment. Hokkanen H. M.T. and Hajeck A.E., Kluwer Academic Pub.: 151-167.	No	No
(Doc. I – Section 2.4.3)	Lacey L.A.,	2007	*Bacillus thuringiensis* serovariety *israelensis* and B*acillus sphaericus* for mosquito control. *Journal of the American Mosquito Control Association* 23(sp2):133-163	No	No
(Doc. II A – Section 1.3)	Glare T.R. and M. O'Callaghan	2000	Bacillus thuringiensis: Biology, Ecology and Safety. John Wiley, N.Y	No	No
(Doc. I – Section 2.4.1 **Doc.IIA - Section 4.1.1)**	Akiba Y.	1986	Microbial Ecology of *Bacillus thuringiensis* VI. Germination of *Bacillus thuringiensis* spores in the soil. *Appl. Ent. Zool.* **21**, 76-80.	No	No

Section No / Reference No	**Author(s)**	**Year**	**Title. Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published**	**Data Protection Claimed (Yes/No)**	**Owner**
(Doc. II A – Section 2.3)	Bravo A., Gill S.S., Soberon M.,	2007	Mode of action of Bacillus thuringiensis Cry and Cyt toxins and their potential for insect control *Toxicon*, **49**(4): 423-435	No	No
(Doc. II A – Section 3.1.1)	De Barjac H. and Sutherland D.J.	1990	Bacterial control of Mosquitoes and Black flies; in *Biochemistry, Genetics and applications of Bacillus thuringiensis and Bacillus sphaericus.* Rutgers University Press.	No	No
(Doc. II A – Section 3.1.1)	Mayes, M.E., Held, G.A., Lau, C., Sely, J.C., Roe, R.M., Dauterman, W.C. and Kawanishi, C.Y.	1989	Characterisation of the Mammalian Toxicity of the Crystal Polypeptides of *Bacillus thuringiensis* subsp. *Israelensis.* *Fundamental and Applied Toxicology* **13**, 310-322	No	No
(Doc. II A – Section 3.1.1)	Cheung, P.Y.K., Roe, R.M., Hammock, B.D., Judson, C.L., and Montague, M.A.	1985	The apparent in vivo neuromuscular effects of the δ-endotoxin of bacillus thuringiensis var israelensis in mice and insects of four orders. *Pesticide Biochemistry and Physiology* **23**, 85-94.	No	No
(Doc. II A – Section 3.1.1)	Siegel, J.P. and Shadduck, J.A.	1990	Clearance of Bacillus sphaericus and Bacillus thuringiensis ssp. israelensis from Mammals. *J. Econ. Entomol*. **83**(2): 347-355	No	No
(Doc. II A – Section 3.1)	Shadduck, J.A.		*Bacillus thuringiensis* serotype H-14 maximum challenge and eye irritation safety tests in mammals	No	No
(Doc. II A – Section 3.1.1)	Siegel J.P., Shadduck J.A.	1990	Clearance of *Bacillus sphaericus* and *Bacillus thuringiensis* ssp. *israelensis* from Mammals. *J. Econ. Entomol*. **83**(2): 347-	No	No

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(Doc. II A – Section 3.1.1)	Shokubutsu Boeki :	1991	Enteropathogenicity of *Bacillus thuringiensis* for humans. 45(12): 18-22	No	No
(Doc. II A – Section 4.1.1)	Martin P.A.W.	1991	Dynamics of *Bacillus thuringiensis* turnover in soil. Proceedings of The General Meeting of the American Society for Microbiolog, p. 315	No	No
(Doc. II A – Section 4.1.1)	Glare T.R. and M. O'Callaghan	2000	Bacillus thuringiensis: Biology, Ecology and Safety. John Wiley, N.Y	No	No
(Doc. II A – Section 4.1.1)	N.B. Hendriksen and B.M. Hansen	2002	Long-term surviaval and germinationof Bacillus thuringiensis var. Kurstaki in a field trial *Can J. Microbiol.* **48**, 256-261	No	No

Section No / Reference No	Author(s)	Year	Title. Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published	Data Protection Claimed (Yes/No)	Owner
(Doc. II A – Section 4.1.1)	Vettori C., Paffetti D., Safena D., Stotzky G. and R. Giannini	2003	Persistence of toxins and cells of *Bacillus thuringiensis* subsp. *kurstaki* introduced in sprays to Sardinia soils. *Soil Biol. Biochem.* **35**, 1635-1642.	No	No
(Doc. II A – Section 4.1.1)	S. De Respinis, A. Demarta, N. Patocchi, P. Luthy, R. Peduzzi and M. Tonella	2006	Molecular identification of *Bacillus thuringiensis* var. *israelensis* to trace its fate after application as a biological insecticide in wetland ecosystems. *Lett. Appl. Microbiol.* **43**, 495-601	No	No
(Doc. II A – Section 4.1.1)	Dong Y., Zhang X., Xu J., Zhang L.	2004	Insecticidal *Bacillus thuringiensis* silences *Erwinia carotovora* Virulence by a New form of microbial antagonism, signal interference. *Microbiology*. **70**, 954-960.	No	No
(Doc. II A – Section 4.1.1)	Hajaij M., Carron A., Deleuze J., Gaven B., Setier-Rio M., Vigo G., Thiéry I., Nielsen-LeRoux C. Lagneau C.	2005	Low Persistence of *Bacillus thuringiensis* Serovar *israelensis* Spores in Four Mosquito Biotopes of a Salt Marsh in Southern France. *Microbial Ecology* **50**, 477-487.	No	No
(Doc. II A – Section 4.1.1)	Martin A.W., Travers R.S.	1989	Worldwide abundance and distribution of *Bacillus thuringiensis* isolates. *Appl. Environ. Microbiol.* **55**, 2437-2442.	No	No
(Doc. II A – Section 4.1.1)	Chilcott C., Wingley P..	1993	Isolation and toxicity of *Bacillus thuringiensis* from soil and insect habitats. *New Zealand. J. Invert. Pathol.* **61**, 244-247	No	No

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(Doc. II A – Section 4.1.1)	DeLucca A.J.I. Simonson J.G. Larson A.D.	1981	*Bacillus thuringiensis* distribution in soil of the United States. *Can. J. Microbiol.* **27**, 865-870.	No	No
(Doc. II A – Section 4.1.1)	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. Environmental Project No. 316. Danish Environmental Protection Agency, Denmark.	No	No
(Doc. II A – Section 4.1.1)	Pedersen J.C., Damgaard P.H., Ellemberg J. Hansen B.M.	1995	Dispersal of *Bacillus thuringiensis* var. *kurstaki* in an experimental cabbage field. *Can. J. Microbiol.* **41**, 118-125	No	No
(Doc. II A – Section 4.1.1)	West A.W., Burges H.D., Dixon T.J. Wyborn C.H.	1985	Survival of *Bacillus thuringiensis* and *Bacillus Cereus* spore inocula in soil: effects of pH, moisture, nutrient availability and indigenous microorganisms. *Soil Biol. Biochem.* **17**, 657-665.	No	No
(Doc. II A – Section 4.1.1)	C.J.H. Pruett, H.D. Burges and C.H. Wyborn	1980	Effect of exposure to soil on potency and spore viability of *Bacillus thuringiensis*. *J. Invert. Pathol.* **35**, 168-174.	No	No
(Doc. II A – Section 4.1.1)	West A.W.	1984	Fate of the insecticidal, proteinaceous parasporal crystal of *Bacillus thuringiensis* in soil. *Soil Biol. Biochem.* **16**, 357-360	No	No
(Doc. II A – Section 4.1.1)	Tapp H.. Stotzky G	1995	Insecticidal activity of the toxins from *Bacillus thuringiensis* subspecies *kurstaky* and *tenebrionis* adsorbed and bound on pure and soil clays. *Appl. Environ. Microbiol.* **61**, 1786-1790	No	No

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(Doc. II A – Section 4.1.1)	Crecchio C. Stotzky G.	1998	Insecticidal activity and biodegradation of the toxin from *Bacillus thuringiensis* subsp. *kurstaki* bound to humic acids from soil. *Soil Biol. Biochem.* 30, 463-470.	No	No
(Doc. II A – Section 4.1.1)	C. Crecchio and G. Stotzky	2001	Biodegradation and insecticidal activity of the toxin from *Bacillus thuringiensis* subsp. *Kurstaki* bound on complexes of montmorillinite-humic acids Al hydoxypolymers. *Soil. Biol. Biochem.* 33, 573-581.	No	No
(Doc. II A – Section 4.1.1)	W. Sheeran and S.W. Fisher	1992	The effect of agitation, sediment, and competition on the persistence and efficacy of *Bt israelensis* (*Bti*). *Ecotox. Environ. Safety* 24, 338-346.	No	No
(Doc. II A – Section 4.1.1)	Menon A.S., Mestral J. De	1985	Survivalof *Bacillus thutingiensis* var. *kurstaki* in water. *Water air soil Pollut*. 25, 265-274.	No	No

Section No / Reference No	**Author(s)**	**Year**	**Title. Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published**	**Data Protection Claimed (Yes/No)**	**Owner**
(Doc. II A – Section 4.1.1)	Forsberg C.W., Henderson M., Henry E. Roberts J.R.	1976	Bt: Its Effects on Environmental Quality.. Publ: National Research Council Canada N° 15383.	No	No
(Doc. IIA - Section 4.1.1)	Goodyear A.	2005	Behaviour of the Microbial Pest Control Agent *Bacillus thuringiensis* subsp *israelensis* in Soil. TSGE report number 22-1-05. SOIL	No	No
(Doc. II A – Section 4.1.1)	Grieco V.M. Spencer K.D.	1978	Inactivation of *Bacillus thuringiensis* spores by ultraviolet and visible light. *Appl. Environ. Microbiol.* **35**, 906-910.	No	No
(Doc. II A – Section 4.1.1)	Myasnik M., Manasherob R., Ben-Dov E., Zaritsky A., Margalith Y., Barak Z.	2001	Comparative sensibility To UV-B radiation of two *Bacillus thuringiensis* subspecies and other *Bacillus* sp. *Curr. Microbiol.* **43**, 140-143.	No	No
(Doc. II A – Section 4.1.1)	M. Pusztai, P. Fast, L. Gringorten, H. Kaplan, T. Lessard, P.R. Carey	1991	The mechanism of sunlight – mediated inactivation of *Bacillus thuringiensis* crystals. *Biochem. J.* **273**, 43-47.	No	No
(Doc. II A – Section 4.1.1)	Teschke K., Chow Y., Bartlett K., Ross A., Van Netten C.	2001	Spatial and temporal distribution of airborne *Bacillus thuringiensis* var *kurstaki* during an aerial spry program for gypsi moth eradication. *Environ. Health Perspective* **109**, 47-54.	No	No

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(Doc. II A – Section 4.1.1)	Menon A.S., De Mestral J.	1985	Survival of *Bacillus thuringiensis* var. *kurstaki* in water. *Water air soil Pollut.* **25**, 265-274	No	No
(Doc. II A – Section 4.2.1)	Balcer M. D., K. I. Schmude J. Snitgen and A. R. Lima.(	1999	Long-term effects of the mosquito control agents Bti (*Bacillus thuringiensis israelensis*) and methoprene on non-target macroinvertebrates in wetlands in Wright County, Minnesota (1997–1998). Report to Metropolitan Mosquito Control District,. St. Paul, Minnesota. 76. plus appendices	No	No
(Doc. II A – Section 4.2.1)	Hershey A.E., Lima A.R., Niemi G.J., Regal R.R.,	1998	Effects of B*acillus thuringiensis israelensis* (bti) and methoprene on nontarget macroinvertebrates in Minnesota wetlands. *Ecological Applications*: **8** (1), 41-60.	No	No
Doc. II A – Section 4.2.1	Koskella, J, Stotzky, G	2002	Larvicidal toxins from *Bacillus thuringiensis* subspp. *kurstaki*, *morrisoni* (strain *tenebrionis*) and *israelensis* have no microbicidal or microbiostatic activity against selected bacteria, fungi, and algae in vitro *Canadian Journal of Microbiology*, 2002, 48, 262 - 267.	No	No

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(Doc. II A – Section 4.2.1)	Pont D., Franquet E., Tourenq J. N.	1999	Impact of different *Bacillus thuringiensis* variety *israelensis* treatments on a chironomid (Diptera: Chironomidae) community in a temporary marsh. *J Econ Entomol* **92**:266–272.	No	No
(Doc. II A – Section 4.2.1)	Tilquin M. Paris M., Reynaud S., Despres L., Ravanel P., Geremia R.A., Gury J.,	2008	Long Lasting Persistence of *Bacillus thuringiensis* Subsp. *israelensis* (*Bti*) in Mosquito Natural Habitats PLoS ONE. 2008; 3(10): e3432. doi:10.1371/journal.pone.0003432 .	No	No
(Doc. II A – Section 4.2.1)	Balcer, M. D., K. I. Schmude, J. Snitgen, and A. R. Lima.	1999	Long-term effects of the mosquito control agents Bti (*Bacillus thuringiensis israelensis*) and methoprene on non-target macroinvertebrates in *Wetlands in Wright County, Minnesota (1997–1998).* Report to Metropolitan Mosquito Control District,. St. Paul, Minnesota. 76. plus appendices.	No	No
(Doc. II A – Section 4.2.1)	Schmude, K. I., Balcer, M. D., Lima, A. R.	1997	Effects of the mosquito control agents *Bti* (*Bacillus thuringiensis israelensis*) and methoprene on non-target macroinvertebrates in *Wetlands in Wright County, Minnesota (1997)*. Report to Metropolitan Mosquito Control District, St. Paul, Minnesota. 28pp. plus appendices.	No	No

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(Doc. II A – Section 4.2.1)	Becker N.	2005	Biological control of mosquitoes: management of the Upper Rhine mosquito population as a model program. In: *An ecological and societal approach to biological control* - Eilenberg J., Hokkanen Heikki M. T. Eds., Chapt.11: Pag.227-245	No	No
(Doc. II A – Section 4.2.1)	Lacey L.A., Merritt R.W.	2003	The safety of bacterial microbial agents used for black fly and mosquito control in aquatic environments. In: *Environment impact of microbial insecticides*. Need and methods for risk assessment. Hokkanen H. M.T. and Hajeck A.E., Kluwer Academic Pub.: 151-167.	No	No
(Doc. II A – Section 4.2.1)	Lacey L.A	2007	*Bacillus thuringiensis* serovariety *israelensis* and B*acillus sphaericus* for mosquito control. *Journal of the American Mosquito Control Association* **23**(2):133-163	No	No
(Doc. II A – Section 4.2.1)	Lundström J.O., Schäfer M.L., Petersson E., Persson Vinnersten T.Z., Landin J., Brodin Y.	2009	Production of wetland Chironomidae (Diptera) and the effects of using ***Bacillus thuringiensis israelensis*** for mosquito control. *Bulletin of Entomological Research*, Published online by Cambridge University Press 05 Jun 2009 doi:10.1017/S0007485309990137	No	No

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(Doc. II A – Section 4.2.1)	Rezza G., L Nicoletti, R Angelini, R Romi, A C Finarelli, M Panning, P Cordioli, C Fortuna, S Boros, F Magurano, G Silvi, P Angelini, M Dottori, M G Ciufolini, G C Majori, A Cassone, for the CHIKV study group	2007	Infection with chikungunya virus in Italy: an outbreak in a temperate region. *Lancet* 2007; 370: 1840–46.	No	No
(Doc. II A – Section 4.2.1)	Genchi C., Rinaldi L., Mortarino M., Genchi M., Cringoli G.,	2009	Climate and Dirofilaria infection in Europe. *Veterinary Parasitology* **163**: 286–292	No	No
(Doc. II A – Section 4.2.1)	Zeller H. G. , Schuffenecker	2004	West Nile Virus: An Overview of Its Spread in Europe and the Mediterranean Basin in Contrast to Its Spread in the Americas. *Eur J Clin Microbiol Infect Dis*. **23**:147–156	No	No

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(Doc. IIB - Section 8.3.1.1)	Goodyear A.	2005	Behaviour of the Microbial Pest Control Agent *Bacillus thuringiensis* subsp *israelensis* in Soil. TSGE report number 22-1-05. SOIL	No	No
(Doc. IIB – Section 8.3.1.1)	Tapp H., Stotzky G.	1995	Insecticidal activity of the toxins from *Bacillus thuringiensis* subspecies *kurstaky* and *tenebrionis* adsorbed and bound on pure and soil clays. *Applied Environmental Microbiology*, **61**(5): 1786-1790	No	No
(Doc. IIB - Section 8.3.1.1)	Crecchio C,. Stotzky G.	1998	Insecticidal activity and biodegradation of the toxin from *Bacillus thuringiensis* subsp. *kurstaki* bound to humic acids from soil. *Soil Biol. Biochem*. **30**,463-470.	No	No
(Doc. IIB - Section 8.3.1.1)	C. Crecchio, G. Stotzky	2001	Biodegradation and insecticidal activity of the toxin from *Bacillus thuringiensis* subsp. *Kurstaki* bound on complexes of montmorillinite-humic acids Al hydoxypolymers. *Soil. Biol. Biochem*. **33**, 573-581.	No	No
(Doc. IIB - Section 8.3.1.1)	A.S. Menon, J. De Mestral	1985	Survivalof *Bacillus thutingiensis* var. *kurstaki* in water. *Water Air Soil Pollut*. **25**, 265-274	No	No

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(Doc. IIB - Section 8.3.1.1)	T.R. Glare, M. O'Callaghan	2000	*Bacillus thuringiensis:* Biology, Ecology and Safety. John Wiley, N.Y.	No	No
(Doc. IIB - Section 8.3.1.1)	FOCUS Working Group	2006	"Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration" Report of the FOCUS Working Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 2.0, 434 pp	No	No
(Doc. IIB - Section 8.3.1.1)	MED-Rice Working Group	2003	Guidance Document for Environmental Risk Assessments of Active Substances used on Rice in the EU for Annex I Inclusion. Document prepared by Working Group on MED-Rice, EU Document Reference SANCO/1090/2000 – rev.1, Brussels, June 2003, 108 pp.	No	No
(Doc. IIC – Section 13)	Hokkanen H. M. T., Hajek A. E., eds.	2001	The safety of bacterial microbial agents used for black fly and mosquito control in aquatic environments, IN: *Environmental Impacts of Microbial Insecticides: Need and Methods for Risk Assessment.* Kluwer Academic Publishers Dordrecht, The Netherlands pp. 151-168.	No	No

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(Doc. IIC – Section 13.1.1)	Mizuki E., Maeda M., Tanaka R., Lee D.-W., Hara M., Akao T., Yamashita S., Kim H. S., Ichimatsu T. Ohba M.	2001	*Bacillua thuringiensis*: A Common Member of Microflora in Activated Sludge of a Sewage Treatment Plant. *Current Microbiology* **42**, 422-425	No	No

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Section 1					
IIIA, 1.3.4/01 Confidential	Smith, R.A., Cooper, R.D.	1990	VectoBac Technical Powder (EPA Registration Number 275-54) Product Chemistry Based on *Bacillus thuringiensis*, subspecies *israelensis* Strain AM65-52 (ATCC-SD-1276) as the Active Ingredient. Abbott Laboratories, unpublished report no. VTP-02. GLP, unpublished.	Y	Valent BioSciences
IIIA, 1.3.4/02	Lecadet, M.-M. *et al*.	1999	Updating the H-Antigen Classification of *Bacillus thuringiensis*. *Journal of Applied Microbiology* 1999, 86, 660-672. Non GLP, published research.	N	No
IIIA, 1.3.4/03	Wie, S. *et al*.	1982	Enzyme-Linked Immunosorbent Assays for Detection and Quantitation of the Entomocidal Parasporal Crystalline Protein of *Bacillus thuringiensis* subsp. *kurataki and israelensis*. *Applied and Environmental Microbiology*, Volume 43, No. 4, April 1982, p.891 to 894. Non GLP, published research.	N	NO-
IIIA, 1.3.4/04 Confidential	Benson, T.	2005	Summary Report Genetic Comparison of *Bacillus thuringiensis* subsp. *israelensis* Strain AM65-52 to other Bacillus Strains using AFLP. Valent BioSciences, report no. not stated. Non GLP, unpublished.	Y	Valent BioSciences
IIIA, 1.4.2.1/01 Confidential	Cooper, R.D., Smith, R.A.	1990	Discussion of the Formation of Unintentional Ingredients. Valent BioSciences, report no. VTP/TE-07. GLP, unpublished.	Y	Valent BioSciences

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IIIA, 1.4.3/01 Confidential	Benzon, G.L.	2002	Analysis of Dipteran Biopotency of Vectobac HP TP (ABG-6164F). Benzon Research, report no. VB0102P. GLP, unpublished.	Y	Valent BioSciences
IIIA, 1.4.3/02 Confidential	Isaacson, J.A.	1991	Analysis of Beta-exotoxin (thuringiensin) Content of Five Lots of Vectobac TP by Housefly Bioassay. Abbott Laboratories, report no. 910-9011. GLP, unpublished.	Y	Valent BioSciences
IIIA, 1.4.3/03 Confidential	Coddens, M.	1990	Vectobac Technical Powder (EPA Registration Number 275-54) Product chemistry Based on Bacillus thuringiensis, subspecies israelensis , Strain AM65-52 (ATCC-SD-12796) as the Active Ingredient. Abbott Laboratories, report no. VTP-03. GLP, unpublished.	Y	Valent BioSciences
IIIA, 1.4.3/04 Confidential	Smith, R.A., Cooper, R.D.	1990	VectoBac Technical Powder (EPA Registration Number 275-54) Product Chemistry Based on *Bacillus thuringiensis*, subspecies *israelensis* Strain AM65-52 (ATCC-SD-1276) as the Active Ingredient. Abbott Laboratories, unpublished report no. VTP-02. GLP, unpublished.	Y	Valent BioSciences
IIIA, 1.4.3/05 Confidential	Brand, R	1998	Bioburden analysis of VectoBac WDG (ABG-6490). Abbott Laboratories report no. 054-97 GLP, unpublished.	Y	Valent BioSciences
Section 2					
IIIA, 2.1.2/01	Martin, P.A.W. Travers, R.S.	1989	Worldwide Abundance and Distribution of *Bacillus thuringiensis* Isolates. *Applied and Environmental Microbiology*, Oct 1989. p. 2437-2442. Non GLP, published research.	N	NO-

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IIIA, 2.1.2/02	Smith, R.A., Couche, G.A..	1991	The Phylloplane as a Source of *Bacillus thuringiensis.* *Applied and Environmental Microbiology*, Jan. 1991. p. 311-315. Non GLP, published research.	N	NO-
IIIA, 2.1.2/03	Meadows, M.P. *et al*.,	1992	Distribution, Frequency, and Diversity of *Bacillus thuringiensis* in an Animal Feed Mill. *Applied and Environmental Microbiology*, Apr. 1992. p 1334-1350. Non GLP, published research.	N	NO-
IIIA, 2.1.2/04	Hansen, B.M. *et al*.	1998	Molecular and Phenotypic Characterisation of *Bacillus thuringiensis* Isolated from Leaves and Insects. *Journal of Invertebrate Pathology* 71, 106 - 114 (1998). Non GLP, published research.	N	NO-
IIIA, 2.3/01 Confidential	Smith, R.A., Cooper, R.D.	1990	VectoBac Technical Powder (EPA Registration Number 275-54) Product Chemistry Based on *Bacillus thuringiensis*, subspecies *israelensis* Strain AM65-52 (ATCC-SD-1276) as the Active Ingredient. Abbott Laboratories, unpublished report no. VTP-02. GLP, unpublished.	Y	Valent BioSciences
IIIA, 2.8/01	Goodyear, A	2005	*Bacillus thuringiensis* subsp. *israelensis*, Strain AM65-52: Lack of Metabolites of Concern Expert Review for EU Dossier. Valent BioSciences, report no. 22-1-5.TOX. Non GLP, unpublished.	Y	Valent BioSciences

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IIIA, 2.8/02	Nair, A.	2005	High Performance Liquid Chromatography Assay for Type I and Type II β-Exotoxin and their Dephosphorylated Variants in 'VectoBac' Slurry. Valent BioSciences, report no. VBC-LG-C-01-02-0005. GLP, unpublished.	Y	Valent BioSciences
IIIA, 2.8/03	Chang, W.	1994	Determination of β-Exotoxin in 'VectoBac' TGAI. Valent BioSciences, report no. 82-2435-62. Non GLP, unpublished.	Y	Valent BioSciences
IIIA, 2.8/04	Bowman, L.	2004	Summary Report: Detection of Enterotoxin in Valent BioSciences Bt Fermentation Beers and Bt Products. Valent BioSciences, report no. not stated. Non GLP, unpublished.	Y	Valent BioSciences
Section 3					
IIIA, 3.4/01 Confidential	Rowell, R.L.	2005	Method of Production and Quality Control for Vectobac Products (*Bacillus thuringiensis* subsp *israelensis*). Valent BioSciences, report no. VBC-03/05-1. GLP, unpublished.	Y	Valent BioSciences
Section 4					
IIIA, 4.1.2/01 Confidential	Coddens, M.	1990	Vectobac Technical Powder (EPA Registration Number 275-54) Product chemistry Based on *Bacillus thuringiensis*, subspecies *israelensis* , Strain AM65-52 (ATCC-SD-12796) as the Active Ingredient. Abbott Laboratories, report no. VTP-03. GLP, unpublished.	Y	Valent BioSciences

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IIIA,4.1.6/01	Nair, A.	2005	High Performance Liquid Chromatography Assay for Type I and Type II β-Exotoxin and their Dephosphorylated Variants in 'VectoBac' Slurry. Valent BioSciences, report no. VBC-LG-C-01-02-0005. GLP, unpublished.	Y	Valent BioSciences
IIIA, 4.1.6/02	Chang, W.	1994	Determination of β-Exotoxin in 'VectoBac' TGAI. Valent BioSciences, report no. 82-2435-62. Non GLP, unpublished.	Y	Valent BioSciences
IIIA, 4.1.6/03	Campbell, D.P., Dieball, D.E., Brackett, J.M.	1987	Rapid HPLC Assay for the beta-exotoxin of *Bacillus thuringiensis*, J. *Agric. Food Chem*. 1987, 35, 156-158. Non GLP, published research.	N	NO-
Section 5					
IIIA, 5.1.2/01	Glynn, S.	2005	Internal Memorandum. *Bacillus thuringiensis* subsp. *israelensis*: employee health effects	Y	Valent Biosciences
IIIA, 5.1.3/01	Not stated.	NA	Patch Testing Report to Agriquality. Available through the New Zealand Health Service	N	Valent Biosciences
IIIA, 5.1.4/01	Pearson, H, E.	1970	Human infections caused by organisms of the Bacillus species. *Am. J. Clin. Path*. 53: 506-515, 1970	N	NO-
IIIA, 5.1.4/02	Shokubutsu Boeki	1991	Enteropathogenicity of *Bacillus thuringiensis* for humans. Tokyo Municipal Research Laboratory of Public Health. 45(12): 18-22, 1991	N	NO-
IIIA, 5.2.1/01	Morris, T.D.	1995	Delayed contact hypersensitivity study in guinea pigs (Buehler Technique) Hill Top Biolabs, Inc., Miamiville, OH, USA. Report No. 94-8488, GLP. Unpublished	Y	Valent Biosciences

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IIIA, 5.2.1/02	Shults, S.K., Brock, A.W. and Laveglia, J.	1995a	Acute oral toxicity (LD_{50}) study in rats with 'VectoBac' Technical. Ricerca, Inc., Painesville, Ohio, USA Report No. 6314-95-0090-TX-001 GLP. Unpublished	Y	Valent Biosciences
IIIA, 5.2.1/03	Shults, S.K.	1995	Acute dermal toxicity (LD_{50}) study in albino rabbits with 'VectoBac' Technical; Ricerca, Inc., Painesville, OH, USA; Report No. 6314-95-0091-TX-001 GLP. Unpublished	Y	Valent Biosciences
IIIA, 5.2.1/04	Shults, S.K., Brock, A.W. and Laveglia, J.	1995 b	Primary Dermal Irritation Study in Albino Rabbits with 'VectoBac' Technical Powder; Ricerca, Inc., Painesville, OH ,USA Report No. 6314-95-0093-TX-001 GLP. Unpublished	Y	Valent Biosciences
IIIA, 5.2.1/05	Shults, S.K., Brock, A.W. and Laveglia, J.	1995c	Primary eye irritation study in albino rabbits with 'VectoBac' Technical Powder; Ricerca, Inc., Painesville, OH, USA Report No. 6314-95-0092-TX-001 GLP. Unpublished	Y	Valent Biosciences
IIIA, 5.2.2.1/01	David, R.M.	1990a	Acute oral toxicity/pathogenicity study of 'VectoBac' Technical Material (*Bacillus thuringiensis* var. *israelensis*) in Rats Microbiological Associates Inc, Bethesda, MD, USA. Report No. G-7264.222 GLP. Unpublished	Y	Valent Biosciences
IIIA, 5.2.2.1/02	Rippel, R.H.	1981a	Effect of Orally Administered *Bacillus thuringiensis* var. *israelensis* on Performance of Young Rat. Abbott Laboratories Chemical and Agricultural Division, Research Center, Long Grove, Illinois, USA Report No. 912-1959, project number 90 71 1 705.03 Non-GLP. Unpublished	Y	Valent Biosciences

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IIIA, 5.2.2.1/03	Shults, S.K., Brock, A.W. and Laveglia, J.	1995 d	Acute oral toxicity (LD_{50}) study in rats with 'VectoBac' Technical. Ricerca, Inc., Painesville, Ohio, USA Report No. 6314-95-0090-TX-001 GLP. Unpublished	Y	Valent Biosciences
IIIA, 5.2.2.2/01	David, R.M.	1990 b	Acute pulmonary toxicity/pathogenicity study of 'VectoBac' Technical Material (*Bacillus thuringiensis* var. *israelensis* in rats Microbiological Associates, Inc., Bethesda, MD, USA Report No. G-7264.225 GLP. Unpublished	Y	Valent Biosciences
IIIA, 5.2.2.2/02	Rippel, R.H.	1981 b	Acute Safety and Infectivity of *Bacillus thuringiensis* var. *israelensis* Administered to Rat via Intratracheal Instillation. Abbott Laboratories Chemical and Agricultural Division, Research Center, Long Grove, Illinois, USA Report No. 90 71 1 705.03 Non-GLP. Unpublished	Y	Valent Biosciences
IIIA, 5.2.2.2/03	Bennick, J.	1996	'VectoBac' Technical Powder Code 43494. Acute inhalation toxicity study in rats Stillmeadow, Inc., Sugar Land, TX; USA. Report No. 1723-94. GLP. Unpublished	Y	Valent Biosciences
IIIA, 5.2.2.3/01	David, R.M.	1990c	Acute intravenous toxicity/pathogenicity study of 'VectoBac' Technical Material (*Bacillus thuringiensis* var. *israelensis*) in rats Microbiological Associates, Inc., Bethesda, MD, USA Report No. G-7264.224 GLP. Unpublished	Y	Valent Biosciences

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IIIA, 5.2.2.3/02	Ferry, E.W.	1990	Intraperitoneal Injection Test with 'VectoBac' Technical Powder Abbott Laboratories, North Chicago, IL, USA Report No. VTP/TE-05 Non-GLP. Unpublished	Y	Valent Biosciences
IIIA, 5.2.3/01	Lawlor, M.A.	1997	Mutagenicity Test with XenTari Technical Powder In The Salmonella-Escherichia Coli/Mammalian-Microsome Reverse Mutation Assay Covance Laboratories Inc., Vienna, Virginia, USA Report No. 18447-0-409 GLP. Unpublished	Y	Valent Biosciences
IIIA, 5.2.5.1/01	Kumar, T.	1994	Subacute Inhalation Toxicity Study of 'VectoBac' 12 AS in Wistar Rats. Fredrick Institute of Plant Protection and Toxicology, Padappai, Tamil Nadu, India Report No. 6779 GLP. Unpublished	Y	Valent Biosciences
IIIA, 5.3/01	Rippel, R.H.	1979	Safety of *Bacillus thuringiensis israelensis* in rabbits. Abbott Laboratories, Chemical and Agricultural Products Division, Long Grove, IL, USA. Report No. 912-1928; project number 90 71 1 700.00 GLP. Unpublished	Y	Valent Biosciences
IIIA, 5.3/02	Rippel, R.H.	1981c	Effect of *Bacillus thuringiensis* var. *israelensis* applied to the abraded epidermis of the rabbit. Abbott Laboratories, Chemical and Agricultural Products Division, Long Grove, IL, USA. Report No. 912-1958; project number 90 71 1 705.03 GLP. Unpublished	Y	Valent Biosciences

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IIIA, 5.3/03	Kandasamy, R., Ramachandran, P.V. and Balakrishna, P.	2000	Subacute oral toxicity study of 'VectoBac' 12 AS in mongrel dogs Fredrick Institute of Plant Protection and Toxicology, Padappai, Tamil Nadu, India. Report No. 6773 GLP. Unpublished	Y	Valent Biosciences
IIIA, 5.3/04	de Barjac, H., Larget, I., Bénichou, L., Cosmao, V., Viviani, G. Ripouteau, H. and Papion, S.	Not dated	Innocuity test on mammals with serotype H-14 of *Bacillus thuringiensis* World Health Organisation Document reference WHO/VBC/80.761 Non-GLP. Published	N	NO-
Section 6	-	-	No study reports submitted	-	-
Section 7					
IIIA, 7.1.1/01	Goodyear, A	2005	Behaviour of the Microbial Pest Control Agent *Bacillus thuringiensis* subsp *israelensis* in Soil. TSGE report number 22-1-05.SOIL. Non GLP, unpublished.	Y	Valent BioSciences
IIIA, 7.1.2/01	Ohana, B., Marglit, J., Barak, Z.	1987	Fate of *Bacillus thuringiensis* subsp israelensis under simulated Field Conditions. *Applied and Environmental Microbiology.*, Apr 1987. p 828-831.	N	NO-
IIIA, 7.1.2/02	Yousten, A. A., Genthner, F.J. Benfield, E.F.	1992	Fate of *Bacillus sphericus* and *Bacillus thuringiensis* serovar *israelensis* in the Aquatic Environment. *Journal of the American Mosquito Control* Association, Vol 8. No. 2. June 1992.	N	NO-
Section 8					

Section No / Reference No	**Author(s)**	**Year**	**Title.** **Source (where different from company)** **Company, Report No.** **GLP (where relevant) / (Un)Published**	**Data Protection Claimed (Yes/No)**	**Owner**
IIIA, 8.1/01	Lattin, A, Grimes, J, Hoxter, K, Smith, G. J.	1990	'VectoBac' Technical Material (*Bacillus thuringiensis* var *israelensis*): An avian oral toxicity and pathogenicity study in the Mallard Wildlife International Ltd., report no. 161-115. GLP, Unpublished.	Y	Valent BioSciences
IIIA, 8.1/02	Lattin, A, Hoxter, K, Smith, G. J.	1990	'VectoBac' Technical Material (*Bacillus thuringiensis* var *israelensis*): An avian oral toxicity and pathogenicity study in the Bobwhite Wildlife International Ltd., report no. 161-114. GLP, Unpublished.	Y	Valent BioSciences
IIIA, 8.2.1/01	Page, J. G.	1981a	Acute static aquatic toxicity study in rainbow trout of *Bacillus thuringiensis* var *israelensis*, lot number 26-261-BD Toxigenics, Inc., report number 410-0561 GLP, Unpublished.	Y	Valent BioSciences
IIIA, 8.2.1/02	Page, J. G.	1981 b	Acute static aquatic toxicity study in bluegill of *Bacillus thuringiensis* var *israelensis*, lot number 26-261-BD Toxigenics, Inc., report number 410-0563 GLP, Unpublished.	Y	Valent BioSciences
IIIA, 8.2.1/03	Christensen, K. P.	1990a	'VectoBac' technical material (*Bacillus thuringiensis* var. *israelensis*) – infectivity and pathogenicity to rainbow trout (*Oncorhynchus mykiss*) during a 32-day static renewal test Springborn Laboratories, Inc., report no. 90-2-3242 GLP, Unpublished	Y	Valent BioSciences

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IIIA, 8.2.1/04	Christensen, K. P.	1990 b	'VectoBac' Technical Material (*Bacillus thuringiensis* var *israelensis*) – Infectivity and pathogenicity to Bluegill sunfish (*Lepomis macrochirus*) during a 30-day static renewal test Springborn Laboratories, Inc., report no. 90-2-3228 GLP, Unpublished	Y	Valent BioSciences
IIIA, 8.2.1/05	Christensen, K. P.	1990c	'VectoBac' Technical Material (*Bacillus thuringiensis* var *israelensis*) – Infectivity and pathogenicity to sheepshead minnow (*Cyprinodon variegatus*) during a 30-day static renewal test Springborn Laboratories, Inc., report no. 90-4-3288 GLP, Unpublished	Y	Valent BioSciences
IIIA, 8.2.2/01	Putt, A.E.	1999	'VectoBac' TP (ABG-6164S) – toxicity to water fleas (*Daphnia magna*) under static-renewal conditions Springborn Laboratories, Inc., report no. 2439.6137 GLP, Unpublished	Y	Valent BioSciences
IIIA, 8.2.2/02	Ward, T. J., Boeri, R. L.	1990	Chronic toxicity of 'VectoBac' technical material (*Bacillus thuringiensis* var. *israelensis*) to the daphnid , *Daphnia magna* EnviroSystems Division Resoure Analysts, Incorporated, report no. 9022-A GLP, Unpublished	Y	Valent BioSciences
IIIA, 8.2.2/03	Christensen, K. P.	1990 d	'VectoBac' Technical Material (*Bacillus thuringiensis* var *israelensis*) – Infectivity and pathogenicity to grass shrimp (*Palaemonetes vulgaris*) during a 31-day static renewal test Springborn Laboratories, Inc., report no. 90-5-3339 GLP, Unpublished	Y	Valent BioSciences

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IIIA, 8.2.2/04	Christensen, K. P.	1991	*Bacillus thuringiensis* var. *israelensis* – infectivity and pathogenicity to mayfly nymphs (*Hexagenia sp*) during an 18-day static renewal test Springborn Laboratories, Inc., report no. 91-3-3700 GLP, Unpublished	Y	Valent BioSciences
IIIA, 8.2.2/05	Chandler, G. T.	1990	Chronic toxicity of *Bacillus thuringiensis* var. *israelensis* technical material to the benthic harpacticoid copepod, *Amphiascus minutus* under static conditions University of South Carolina, report no. USC-SPH-2-90 GLP, Unpublished	Y	Valent BioSciences
IIIA, 8.2.2/06	Hershey, A. E., Shannon, L., Axler, R., Ernst, C., Mickelson, P.	1995	Effects of methoprene and *Bti (Bacillus thuringiensis* var *.israelensis)* on non-target insects *Hydrobiologia* 308: 219 - 227 (1995) Non GLP, Published research	N	NO-
IIIA, 8.2.2/07	Merritt, R. W., Walker, E. D., Wilzbach, M. A., Cummins, K. W., Morgan, W. T.	1989	A broad evaluation of B.T.I. for black fly (Diptera:Simulidae) control in a Michigan River: Efficacy, carry and nontarget effects on invertebrates and fish. *Journal of the American Mosquito Control Association* Vol. 5, No. 3, 397 – 415 (1989). Non GLP, Published research	N	NO-
IIIA, 8.2.3/01	Koskella, J, Stotzky, G	2002	Larvicidal toxins from *Bacillus thuringiensis* subspp. *kurstaki*, *morrisoni* (strain *tenebrionis*) and *israelensis* have no microbicidal or microbiostatic activity against selected bacteria, fungi, and algae in vitro *Canadian Journal of Microbiology*, 2002, 48, 262 - 267. Non GLP, Published research.	N	NO-

Section No / Reference No	**Author(s)**	**Year**	**Title.** **Source (where different from company)** **Company, Report No.** **GLP (where relevant) / (Un)Published**	**Data Protection Claimed (Yes/No)**	**Owner**
IIIA, 8.3/01	Atkins, E. L.	1990	Bee adult toxicity feeding test/chronic evaluating the comparative chronic stomach poison toxicity of *Bacillus thuringiensis* subsp. *israelensis* (*Bti*) to honey bee worker adults *Apis mellifera* L. University of California, *Bti* BATFT/C 90-833-FC GLP, Unpublished	Y	Valent BioSciences
IIIA, 8.5/01	Rodgers, M	2006	*Bacillus thuringiensis* subsp. *israelensis* toxicity and pathogenicity to the earthworm. Huntingdon Life Sciences Ltd., Draft Report No.: ZAB 0069/062301 GLP, Unpublished	Y	Valent BioSciences
IIIA, 8.5/02	Benz, G., Altwegg, A.	1974	Safety of *Bacillus thuringiensis* for earthworms *Journal of Invertebrate Pathology* 26, 125 – 126 (1975) Non GLP, Published research.	N	NO-
Section 9	-	-	No study reports submitted	-	-
Section 10	-	-	No study reports submitted	-	-

Section No / Reference No	**Author(s)**	**Year**	**Title.** **Source (where different from company)** **Company, Report No.** **GLP (where relevant) / (Un)Published**	**Data Protection Claimed (Yes/No)**	**Owner**
Section 1	-	-	No study reports submitted	-	-
Section 2					
IIIB, 2.1/01	Young, S.	2003	'VectoBac' WG two year storage stability. Huntingdon Life Sciences Ltd., report no. ABT 401/024588. GLP, unpublished.	Y	Valent Biosciences
IIIB, 2.2/01	Young, S.	2003	'VectoBac' WG two year storage stability. Huntingdon Life Sciences Ltd., report no. ABT 401/024588. GLP, unpublished.	Y	Valent Biosciences
IIIB, 2.3/01 Confidential	Curl, M.G.	2005a	Expert statement on the explosive properties of 'VectoBac' WG formulated preparation. TSGE report no. 22-1-05.EXP. Non-GLP, unpublished	Y	Valent Biosciences
IIIB, 2.3/02 Confidential	Curl, M.G.	2005 b	Expert statement on the oxidising properties of 'VectoBac' WG formulated preparation. TSGE report no. 22-1-05.OXP. Non-GLP, unpublished	Y	Valent Biosciences
IIIB, 2.4/01 Confidential	Curl, M.G.	2005c	Expert statement on the flammability of 'VectoBac' WG formulated preparation. TSGE report no. 22-1-05.FLM. Non-GLP, unpublished	Y	Valent Biosciences
IIIB, 2.5/01	Young, S.	2003	'VectoBac' WG two year storage stability. Huntingdon Life Sciences Ltd., report no. ABT 401/024588. GLP, unpublished.	Y	Valent Biosciences
IIIB, 2.7.1/01	Young, S.	2003	'VectoBac' WG two year storage stability. Huntingdon Life Sciences Ltd., report no. ABT 401/024588. GLP, unpublished.	Y	Valent Biosciences
IIIB, 2.7.2/01	Young, S.	2003	'VectoBac' WG two year storage stability. Huntingdon Life Sciences Ltd., report no. ABT 401/024588. GLP, unpublished.	Y	Valent Biosciences

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IIIB, 2.7.3/01	Young, S.	2003	'VectoBac' WG two year storage stability. Huntingdon Life Sciences Ltd., report no. ABT 401/024588. GLP, unpublished.	Y	Valent Biosciences
IIIB, 2.7.4/01	Young, S.	2003	'VectoBac' WG two year storage stability. Huntingdon Life Sciences Ltd., report no. ABT 401/024588. GLP, unpublished.	Y	Valent Biosciences
IIIB, 2.7.5/01	Young, S.	2003	'VectoBac' WG two year storage stability. Huntingdon Life Sciences Ltd., report no. ABT 401/024588. GLP, unpublished.	Y	Valent Biosciences
Section 3	-	-	No study reports submitted	-	-
Section 4	-	-	No study reports submitted	-	-
Section 5					
IIIB, 5.1/01	Young, S.	2003	'VectoBac' WG two year storage stability. Huntingdon Life Sciences Ltd., report no. ABT 401/024588. GLP, unpublished.	Y	Valent Biosciences
Section 6					
IIIB, 6.1/01	DeChant, P.	2005	A trial to Evaluate 'VectoBac' WDG in low volume ground application for control of container breeding species. Valent BioSciences. Report number 2003PDECH008. Dated 31.1.2005. Non-GLP. Unpublished.	Y	Valent Biosciences
IIIB, 6.1/02	DeChant, P.	2006	A trial to evaluate the efficacy of aerially applied 'VectoBac' WDG for the control of *Ochlerotatus caspius* and *Culex spp.* larvae in rice fields under mid season rice growing conditions. Valent BioSciences. Report number 2003PDECH014. Dated 7.2.2006. Non-GLP. Unpublished.	Y	Valent Biosciences

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IIIB, 6.1/03	Muller, M.J.	1999	Testing of 'VectoBac' WDG Mosquito Control. Valent BioSciences. Report number 7627. Dated 8.2.1999. Non-GLP. Unpublished	Y	Valent Biosciences
IIIB, 6.1/04	Rath, A.	2000a	Report on aerial trial of 'VectoBac' WG. Brisbane City Council, Mosquito and Pest Services, Queensland, Australia. Report number 7667. Dated 02.2000. Non-GLP. Unpublished	Y	Valent Biosciences
IIIB, 6.1/05	DeChant, P.	1999	'VectoBac' WDG comparison to 'VectoBac' 12AS for control of *Aedes vexans*.. Abbot laboratories, Portland OR. Report number 16574. Dated 12.1999. Non-GLP. Unpublished	Y	Valent Biosciences
IIIB, 6.1/06	Ballaux, J.C.	2001	A trial to compare the new 'VectoBac' WDG formulation with the reference 'VectoBac' 12AS for the control of *Aedes caspius* in the estuary of the Odiel river (Huelva). Estuario del Rio Odiel, Huelva, Spain. Report number 2000JBALL006. Dated 07.2001 Non-GLP. Unpublished	Y	Valent Biosciences
IIIB, 6.1/07	DeChant, P.	2001	A trial to conduct large scale field trials with IcyBac delivery system to test effectiveness of this application methodology. Valent Biosciences. Report number 2000PDECH578. Dated 01.25.2002. Non-GLP. Unpublished	Y	Valent Biosciences
IIIB, 6.1/08	Su, T. and Mulla, M.S.	1999	Field evaluation of new water dispersible granular formulations of *Bacillus thuringiensis* Ssp. *Israelensi and Bacillus sphaericus* against *Culex* mosquitoes in microcosms. Department of Entomology. University of California, Riverside. *Journal of the American Mosquito Control Association*. 15(3):356-365, 1999. Non-GLP. Published.	N	NO-

Section No / Reference No	**Author(s)**	**Year**	**Title.** **Source (where different from company)** **Company, Report No.** **GLP (where relevant) / (Un)Published**	**Data Protection Claimed (Yes/No)**	**Owner**
IIIB, 6.1/09	Merritt, R. W., Walker, E. D., Wilzbach, M. A., Cummins, K. W., Morgan, W. T.	1989	A broad evaluation of B.T.I. for black fly (Diptera:Simulidae) control in a Michigan River: Efficacy, carry and nontarget effects on invertebrates and fish. *Journal of the American Mosquito Control Association* Vol. 5, No. 3, 397 – 415 (1989). Non GLP, Published research	N	NO-
IIIB, 6.1/10	Bartninkaitė, I, Bernotienė, R. Pakalniškis, S., Žygutienė, M.	Not known	Bloodsucking blackflies (diptera: simuliidae) and a way to solve the problem. Journal not stated. Non GLP, Published research	N	NO-
IIIB, 6.1/11	Fusco, R.	1996	Evaluation of VectoBac 12AS against *Psychoda alternata* in a Pennsylvania sewer treatment plant utilising plastic media trickling filters. Valent BioSciences report number 1996RFUSC245. Non GLP, Unpublished	Y	Valent Biosciences
IIIB, 6.1/12	Fusco, R.	1995	Opperational use of VectoBac 12AS against *Psychoda spp* in a Pennsylvania sewer treatment plant utilising rock media trickling filters. Valent BioSciences report number 1995RFUSC246. Non GLP, Unpublished	Y	Valent Biosciences
IIIB, 6.1/13	Coombs, R.M., Dancer, B.N., Davies, D.H., Housten, J. and Learner, M.A.	1991	The Use of *Bacillus thuringiensis* var *israelensis* to Control the Nuisance Fly *Sylvicola fenestralis anisopodidae* in Sewage Filter Beds. Water Research 25 (5) 1991. 605-612. Non GLP, Published research	Y	Valent Biosciences
IIIB, 6.1/14	Housten, J., Dancer, B.N. and Learner, M.A.	1991	Control of Sewage Filter flies Using *Bacillus thuringiensis* var *israelensis* Full Scale Trials. Water Research 23 (3) 1989. 379-386. Non GLP, Published research	Y	Valent Biosciences

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IIIB, 6.2/01	DeChant, P.	2002	A trial Determine susceptibility of *Oc. japonicus* 'VectoBac' WDG and VectoLex WDG. Valent Biosciences. Unpublished report number 2001PDECH017. Dated 06.04.2002. Non-GLP. Unpublished	Y	Valent Biosciences
IIIB, 6.2/02	Russell, T.L., Brown, M.D., Purdie, D.M., Ryan, P.A., Kay, B.H.	2003	Efficacy of 'VectoBac' (Bacillus thuringiensis variety israelensis) Formulations for Mosquito Control in Australia. *J. Econ. Entomol.* 96(6): 1786-1791 Non-GLP. Published.	N	NO-
IIIB, 6.2/03	Rath, A.	2000 b	Laboratory study on 'VectoBac' water dispersible granules (WDG) (ABG6511) in comparison with liquid formulation 'VectoBac' 12AS using earthen jar method against vector mosquitoes in the tropical environment. Vector Control Research Unit, University Sains Malaysia, Penang, Malaysia. Report number 12668. Dated 06.2000. Non-GLP. Unpublished	Y	Valent Biosciences
Section 7					
IIIB, 7.1.1/01	Shults, S.K. and Watson, M.	1997a	Acute oral toxicity (LD50) study in rats with 'VectoBac' WDG; Ricerca, Inc., Painesville, Ohio, USA. Report No. 7253-97-0111-TX-001, GLP. Unpublished	Y	Valent Biosciences
IIIB, 7.1.2/01	Bennick, J.	1997	'VectoBac' WDG (ABG-6490) Lot 30-058-BR, Acute Inhalation Toxicity Study in Rats, Stillmeadow, Inc., Sugar Land, Texas, USA. Report No. 3567-97, GLP. Unpublished	Y	Valent Biosciences
IIIB, 7.1.3/01	Shults, S.K. and Watson, M.	1997 b	Acute dermal toxicity (LD50) study in Albino Rabbits with 'VectoBac' WDG (ABG-6490); Ricerca, Inc., Painesville, Ohio, USA. Report No. 7253-97-0112-TX-001, GLP. Unpublished	Y	Valent Biosciences

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IIIB, 7.2.1/01	Shults, S.K. and Watson, M.	1997c	Primary Dermal Irritation Study in Albino Rabbits with 'VectoBac' WDG (ABG-6490); Ricerca, Inc., Painesville, Ohio, USA. Report No. 7253-97-0114-TX-001, GLP. Unpublished	Y	Valent Biosciences
IIIB, 7.2.2/01	Shults, S.K. and Watson, M.	1997d	Primary Eye Irritation Study in Albino Rabbits with 'VectoBac' WDG (ABG-6490); Ricerca, Inc., Painesville, Ohio, USA. Report No. 7253-97-0113-TX-001, GLP. Unpublished	Y	Valent Biosciences
IIIB, 7.2.3/01	Kuhn, J.O.	2001	ABG-6511, 'VectoBac' WDG, Lot 60-068-BR. Guinea pig maximization test for topically applied test substance. Stillmeadow, Inc., Sugarland, Texas, USA Report No. 6281-01, GLP. Unpublished	Y	Valent Biosciences
Section 8	-	-	No study reports submitted	-	-
Section 9	-	-	No study reports submitted	-	-
Section 10					
IIIB, 10.3/01	Bocksch, S	2006	Assessment of side effects of Vectobac WG to the honey bee *Apis mellifera* L. in the laboratory limit test. GAB Biotechnologie GmbH, Report No: 20061012/S1-BLEU GLP. Unpublished	Y	Valent Biosciences
Section 11	-	-	No study reports submitted	-	-
Section 12	-	-	No study reports submitted	-	-