CONCLUSION ON PESTICIDE PEER REVIEW

Conclusion on the peer review of the pesticide risk assessment of the active substance *Bacillus thuringiensis israelensis* AM65-52[1]

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ABSTRACT

The conclusions of the European Food Safety Authority (EFSA) following the peer review of the initial risk assessments carried out by the competent authority of the rapporteur Member State Italy, for the pesticide active substance *Bacillus thuringiensis israelensis* AM65-52 are reported. The context of the peer review was that required by Commission Regulation (EC) No 2229/2004, as amended by Commission Regulation (EC) No 1095/2007. The conclusions were reached on the basis of the evaluation of the representative uses of *Bacillus thuringiensis israelensis* AM65-52 as an insecticide on ornamentals. The reliable endpoints concluded as being appropriate for use in regulatory risk assessment, derived from the available studies and literature in the dossier peer reviewed, are presented. Missing information identified as being required by the regulatory framework is listed. Concerns are identified.

KEY WORDS

Bacillus thuringiensis israelensis AM65-52 peer review, risk assessment, pesticide, insecticide

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SUMMARY

Bacillus thuringiensis israelensis AM65-52 is one of the 295 substances of the fourth stage of the review programme covered by Commission Regulation (EC) No 2229/2004, as amended by Commission Regulation (EC) No 1095/2007.

Bacillus thuringiensis israelensis AM65-52 was included in Annex I to Directive 91/414/EEC on 8 December 2008 pursuant to Article 24b of the Regulation (EC) No 2229/2004 (hereinafter referred to as 'the Regulation') and has subsequently been deemed to be approved under Regulation (EC) No 1107/2009, in accordance with Commission Implementing Regulation (EU) No 540/2011, as amended by Commission Implementing Regulation (EU) No 541/2011. In accordance with Article 25a of the Regulation, as amended by Commission Regulation (EU) No 114/2010, the European Food Safety Authority (EFSA) is required to deliver by 31 December 2012 its view on the draft review report submitted by the European Commission in accordance with Article 25(1) of the Regulation. This review report was established as a result of the initial evaluation provided by the designated rapporteur Member State in the Draft Assessment Report (DAR). The EFSA therefore organised a peer review of the DAR. The conclusions of the peer review are set out in this report.

Italy being the designated rapporteur Member State submitted the DAR on *Bacillus thuringiensis israelensis* AM65-52 in accordance with the provisions of Article 22(1) of the Regulation, which was received by the EFSA on 28 November 2007. The peer review was initiated on 18 April 2008 by dispatching the DAR for consultation of the notifier Sumitomo Chemical Agro Europe SAWS. Subsequently the DAR was distributed on 11 June 2008 for consultation of the Member States. Following consideration of the comments received on the DAR, it was concluded that there was no need to conduct an expert consultation and EFSA should deliver its conclusions on *Bacillus thuringiensis israelensis* AM65-52.

The conclusions laid down in this report were reached on the basis of the evaluation of the representative uses of *Bacillus thuringiensis israelensis* AM65-52 as an insecticide on ornamentals as proposed by the notifier. Full details of the representative uses can be found in Appendix A to this report.

In the area of identity of the micro-organism/biological properties/physical and technical properties and methods of analysis the main data gaps are related to contaminating microorganisms, method for parasporal body protein, identification method, method of analysis for contaminating microorganisms, method for the microorganism, biopotency method, optimal environmental growing conditions and the temperature range of growth, validation of the methods for the toxins and validation for the method for the CFU in the formulation.

In the area of mammalian toxicology, two data gaps were identified. The first one is related to the production of toxins after application, and the risk assessment cannot be finalised for humans that might be exposed to these toxins (e.g. re-entry workers). The second one is related to the validation of the bridging of toxicological data between the formulation VectoBac 12 AS (of unknown composition) and the representative formulation VectoBac WDG.

The only use for this organism is on ornamentals and no edible crops will be treated, therefore a consumer risk assessment is not necessary.

No information has been provided in relation to potential interferences of *Bacillus thuringiensis* with the analytical systems for the control of the quality of drinking water provided for in Directive 98/83/EC. No information has been provided on the potential transfer of genetic material from *Bacillus thuringiensis* to other organisms. Data gaps have been identified and these issues cannot be finalized. The original scientific papers quoted in the fate section of the dossier have not been provided. Therefore, a data gap has been identified. During the peer review a data gap was identified for the groundwater exposure assessment of the crystalline proteins and conversion products that

retain any insecticidal activity. Nevertheless, since only uses in greenhouse on potted plants are intended, adequate management to prevent natural soil and groundwater contamination may be proposed in absence of further information.

A data gap was identified in the Ecotoxicology section i.e. to provide information as regards the risk to biological methods for sewage treatment plants.

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BACKGROUND

Bacillus thuringiensis israelensis AM65-52 is one of the 295 substances of the fourth stage of the review programme covered by Commission Regulation (EC) No 2229/2004,[3] as amended by Commission Regulation (EC) No 1095/2007.[4]

Bacillus thuringiensis israelensis AM65-52 was included in Annex I to Directive 91/414/EEC[5] on 8 December 2008 pursuant to Article 24b of the Regulation (EC) No 2229/2004 (hereinafter referred to as 'the Regulation') and has subsequently been deemed to be approved under Regulation (EC) No 1107/2009,[6] in accordance with Commission Implementing Regulation (EU) No 540/2011,[7] as amended by Commission Implementing Regulation (EU) No 541/2011.[8] In accordance with Article 25a of the Regulation, as amended by Commission Regulation (EU) No 114/2010[9] the European Food Safety Authority (EFSA) is required to deliver by 31 December 2012 its view on the draft review report submitted by the European Commission in accordance with Article 25(1) of the Regulation (European Commission, 2008). This review report was established as a result of the initial evaluation provided by the designated rapporteur Member State in the Draft Assessment Report (DAR). The EFSA therefore organised a peer review of the DAR. The conclusions of the peer review are set out in this report.

Italy being the designated rapporteur Member State submitted the DAR on *Bacillus thuringiensis israelensis* AM65-52 in accordance with the provisions of Article 22(1) of the Regulation, which was received by the EFSA on 28 November 2007 (Italy, 2007). The peer review was initiated on 18 April 2008 by dispatching the DAR for consultation of the notifier Sumitomo Chemical Agro Europe SAS. Subsequently the DAR was distributed on 11 June 2008 for consultation of the Member States. In addition, the EFSA conducted a public consultation on the DAR. The comments received were collated by the EFSA and forwarded to the RMS for compilation and evaluation in the format of a Reporting Table. The comments were evaluated by the RMS in column 3 of the Reporting Table.

The scope of the peer review was considered in a telephone conference between the EFSA, the RMS, and the European Commission on 22 June 2012. On the basis of the comments received and the RMS' evaluation thereof it was concluded that there was no need to conduct an expert consultation.

The outcome of the telephone conference, together with EFSA's further consideration of the comments is reflected in the conclusions set out in column 4 of the Reporting Table. All points that were identified as unresolved at the end of the comment evaluation phase and which required further consideration, and additional information to be submitted by the notifier, were compiled by the EFSA

[3] Commission Regulation (EC) No 2229/2004 of 3 December 2004 laying down further detailed rules for the implementation of the fourth stage of the programme of work referred to in Article 8(2) of Council Directive 91/414/EEC. OJ L 379, 24.12.2004, p.13-63.
[4] Commission Regulation (EC) No 1095/2007 of 20 September 2007 amending Regulation (EC) No 1490/2002 laying down further detailed rules for the implementation of the third stage of the programme of work referred to in Article 8(2) of Council Directive 91/414/EEC and Regulation (EC) No 2229/2004 laying down further detailed rules for the implementation of the fourth stage of the programme of work referred to in Article 8(2) of Council Directive 91/414/EEC. OJ L 246, 21.9.2007, p.19-28.
[5] Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market. OJ L 230, 19.8.1991, p. 1-32, as last amended.
[6] Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. OJ L 309, 24.11.2009, p.1-50.
[7] Commission Implementing Regulation (EU) No 540/2011 of 25 May 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards the list of approved active substances. OJ L 153, 11.6.2011, p.1-186.
[8] Commission Implementing Regulation (EU) No 541/2011 of 1 June 2011 amending Implementing Regulation (EU) No 540/2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards the list of approved active substances. OJ L 153, 11.6.2011, p.187-188.
[9] Commission Regulation (EU) No 114/2010 of 9 February 2010 amending Regulation (EC) No 2229/2004 as regards the time period granted to EFSA for the delivery of its view on the draft review reports concerning the active substances for which there are clear indications that they do not have any harmful effects. OJ L 37, 10.2.2010, p.12.

in the format of an Evaluation Table. The notifier was invited to respond to the comments in column 2 of the Evaluation Table.

The conclusions arising from the consideration by the EFSA, and as appropriate by the RMS, of the points identified in the Evaluation Table, together with the outcome of the expert discussions where these took place, were reported in the final column of the Evaluation Table.

A final consultation on the conclusions arising from the peer review of the risk assessment took place with Member States via a written procedure in November 2012.

This conclusion report summarises the outcome of the peer review of the risk assessment on the active substance and the representative formulation evaluated on the basis of the representative uses as a insecticide on ornamentals, as proposed by the notifier. A list of the relevant end points for the active substance as well as the formulation is provided in Appendix A. In addition, a key supporting document to this conclusion is the Peer Review Report, which is a compilation of the documentation developed to evaluate and address all issues raised in the peer review, from the initial commenting phase to the conclusion. The Peer Review Report (EFSA, 2012) comprises the following documents, in which all views expressed during the course of the peer review, including minority views, can be found:

- the comments received on the DAR,
- the Reporting Table (20 July 2009)
- the Evaluation Table (10 December 2012)
- the report(s) of the scientific consultation with Member State experts (where relevant),
- the comments received on the draft EFSA conclusion.

Given the importance of the DAR including its addendum (compiled version of November 2012 containing all individually submitted addenda (Italy, 2012)) and the Peer Review Report, both documents are considered respectively as background documents A and B to this conclusion.

THE ACTIVE SUBSTANCE AND THE FORMULATED PRODUCT

THE IDENTITY OF THE MICROORGANISM AND THE PROPERTIES OF THE FORMULATED PRODUCT

Bacillus thuringiensis subsp. israelensis strain AM65-52 was isolated from mosquito larvae in Israel.

The representative formulation evaluated is 'VectoBac WG'. The formulation contains approximately 374 g/kg fermentation solids and soluble. This gives approximately 3 x 10^9 CFU/g

The representative use evaluated is on ornamentals as a foliar spray to control fungus gnats *Diptera Sciaridae*. Full details of the GAP can be found in the list of end points in Appendix A.

CONCLUSIONS OF THE EVALUATION

1. Identity of the microorganism/biological properties/physical and technical properties and methods of analysis.

The production culture of *Bacillus thuringiensis* subsp. *israelensis* strain AM65-52, has been placed in the American Type Culture Collection under the accession number ATCC – 1276.

The strain is not a human pathogen but it is related to human pathogens. A data gap was identified for its growth temperature range as well as the optimal growing conditions.

It has been demonstated that this strain does not produce significant quantities of enterotoxins, β-exotoxin and cytolytic proteins in the production process. The content of contaminating micro-organisms was not fully addressed and a data gap was identified.

A method of analysis to unequivocally identify the organism down to strain level was not available to the peer review. All the methods of analysis were not validated for the strain, contaminating and pathogenic micro-organisms and further validation is needed for the method for the toxins.

Methods of analysis for products of plant and animal origin are not necessary as there are no edible crop uses. Methods of analysis for body fluids and tissues are not required as there is no classification for toxic or very toxic.

Environmental methods of analysis may be required in the future once the outstanding environmental data issues have been addressed.

It should be noted that during the shelf-life study there is a 20 % decrease in potency after the two year storage period.

2. Mammalian toxicity

Medical data and sensitisation

Epidemiological data specific to *Bacillus thuringiensis* subsp. *israelensis* AM65-52 were not provided in the dossier. No adverse effects were reported among manufacturing plant personnel exposed to *Bacillus thuringiensis israelensis (Bti)*.

Human patch testing with *Bacillus thuringiensis* subsp. *kurstaki* revealed no cases of contact hypersensitivity whereas VectoBac (containing *Bti* AM65-52) was shown to be skin sensitiser in guinea pigs (Buehler test). Considering that all microbials should be regarded as potential sensitisers, the agreed warning phrase is "Micro-organisms may have the potential to provoke sensitising reactions".

As mentioned by EFSA in an assessment of *Bacillus* bacteria with respect to a Qualified Presumption of Safety (EFSA, 2007), cases of human illness may have been underreported because of the close

relationship to the human pathogen *Bacillus cereus* (*Bc*). Only the production of insecticidal crystal proteins by *Bacillus thuringiensis (Bt)* distinguishes these two species, and many strains of *Bt* produce the same enterotoxins known from *Bc* to cause diarrhoea in humans. Additionally, rodents may not be the best model for addressing the potential of foodborne poisoning related to the *Bacillus cereus* type toxins (enterotoxins) susceptible to be produced by *Bacillus thuringiensis* (EFSA, 2008).

Toxicity studies

For *Bti* strain AM65-52, no detailed analysis of the batches used in the toxicological studies is available. However, further information can be considered as not required, provided that adequate quality control is undertaken on the batches produced, certifying that toxicologically relevant pathogenic microbial contaminants are kept below levels internationally recognised for microbial contaminants (see data gap in section 1).

No information has been provided on the potential transfer of genetic material from *Bti* to other organisms. Data or assessment to demonstrate that this transfer does not occur or in case of occurring will not lead to adverse effects has to be provided.

Most of the available acute toxicity studies were performed with VectoBac preparations (containing *Bacillus thuringiensis* subsp. *israelensis AM65-52)*. No adverse effects were observed in rats after acute oral exposure and the clearance was complete after 21 days. After intratracheal administration, the observed clinical signs and lung lesions were attributed to the presence of foreign material rather than to infectivity or pathogenicity of the microbial. After intravenous or intraperitoneal exposure, no signs of toxicity were observed. No systemic adverse effects were observed in rabbits after dermal exposure, whereas a mild skin and eye irritation was reported.

Considering that no validated method for genotoxicity testing of microorganisms is available, the need for further assessment will have to be considered if human exposure to toxins cannot be excluded. Therefore, a data gap has been identified for an assessment of the production of toxins after application: enterotoxins (*B. cereus* type: haemolytic, non haemolytic or cytotoxic), beta-exotoxin (ATP analogue), cytolytic proteins (parasporins acting in combination with insecticidal crystal proteins).

The available short term studies were performed with the preparation VectoBac 12 AS (of unknown composition). In a 14-day inhalation study (nose-only, 4h/day), no adverse effect was observed in the rats administered 1.84×10^6 spores/L. In a 90-day dog study (oral gavage), the NOAEL was 5×10^6 spores/animal in the absence of treatment-related adverse effect. A justification has to be provided for the bridging of these toxicological data towards the representative formulation VectoBac WDG ABG-6511 (data gap).

Reference values

The derivation of reference values was not considered needed since the micro-organism was not shown to be pathogenic or infective based on the available data and studies.

It is noted that there is a potential of food borne poisoning, related to the *Bacillus cereus* type toxins (enterotoxins) susceptible to be produced by *Bacillus thuringiensis* subsp. *israelensis*. In most instances, food borne diseases caused by *Bacillus cereus* were associated with the intake of 10^5 to 10^8 CFU/g food, whereas lower numbers were reported in some outbreaks (10^3 to 10^4 CFU/g food).

Exposure estimates

Since reference values are not necessary, no exposure estimates are required for the microorganism. Due to the data gap for analysis of the potential toxins (e.g. enterotoxins, beta-exotoxin, cytolytic protein) produced after application, the risk assessment cannot be finalised for the re-entry workers.

3. Residues

The only use for this organism is on ornamentals and no edible crops will be treated, therefore a consumer risk assessment is not necessary.

4. Environmental fate and behaviour

4.1. Fate and behaviour in the environment of the microorganism

Only a review summary of six scientific literature studies has been presented in the dossier. The original papers quoted there have not being made available in the dossier. Therefore, a data gap for scientific literature quoted by the applicant in its assessment has been identified.

No information has been provided in relation to potential interferences of *Bacillus thuringiensis* with the analytical systems for the control of the quality of drinking water provided for in Directive 98/83/EC (See specific Annex VI decision making criteria in Directive 2005/25/EC) (data gap).

No information has been provided on the potential transfer of genetic material from *Bacillus thuringiensis* to other organisms. However, bacilli are known to have the capacity to transfer genetic material trough exchange of plasmids. Therefore, data or assessment to demonstrate that this transfer does not occur or in case of occurring will not lead to unacceptable effects on the environment has to be provided (See specific Annex VI decision making criteria in Directive 2005/25/EC) (data gap).

No studies on **persistence and multiplication in soil** of *Bacillus thuringiensis* subsp. *israelensis* strain AM65-52 are available. Assessment presented in the DARs is based on the scientific publications summarized by the applicant in the dossier on other strains and or species of the *Bacillus thuringiensis*. The experts in the M03 meeting agreed using non strain specific data is acceptable if a worst case end point is selected among the data available over a range of strains. During the peer review a number of issues have been considered to require further clarification or data.

According the information provided by the applicant, vegetative cells are not expected to occur in the marketed products of *Bacillus thuringiensis* subsp. *israelensis* strain AM65-52. Vegetative cells are expected to survive and multiply in soil and dead insect cadavers under favourable conditions. The persistence and multiplication of vegetative cells in soil is assumed to occur only under a narrow range of favourable conditions and limited in time. However, details on the soils and experimental conditions used as well as on the individual measurements performed would be needed to allow predicting the distribution fate and behaviour in soil of the microorganism and the time courses involved.

Spores are one of the main active components of the marketed products of *Bacillus thuringiensis* subsp. *israelensis* strain AM65-52. Spores are formed by sporulation and lysis of vegetative cells. Spores do not multiply but may remain in a latent form for long periods under a variety of environmental conditions. Available data suggest that spores may remain in soil from months to years under field conditions. It is believed that exposure to light (UV and visible) is the factor more strongly affecting its persistence. Exposure to light may be very variable depending on the mode of application and agricultural practices. An estimation of background levels of spores and the time needed to recover this level after application of *Bacillus thuringiensis* subsp. *israelensis* strain AM65-52 is currently not available.

PEC soil values presented in the DARs were not agreed during the peer review, since interception factors calculated for chemicals are not necessary applicable to microorganisms. RMS was requested to provide new PEC soil based assuming no interception (see open point 8.10). This has been calculated and may be found in the corresponding appendix.

No studies on **persistence and multiplication in water** of *Bacillus thuringiensis* subsp. *israelensis* strain AM65-52 are available. Assessment presented in the DAR is based on the summary of scientific

publications submitted by the applicant in the dossier on other strains and or species of the *Bacillus thuringiensis*. However, no individual assessment of the publications was presented in the DAR, so it is difficult to track back the statements and conclusions to the original scientific data. The experts at PRAPeR meeting M3 agreed that no further data would be needed if the aquatic risk assessment could be finalised using an annual total dose PEC_{SW}. PECsurface water calculations provided in Addendum Appendix 3 were not appropriate as they used field spray drift. Therefore, EFSA performed new PEC in surface water for the envisaged greenhouse use. The results can be found in Appendix A.

4.2. Fate and behaviour in the environment of any relevant metabolite formed by the microorganism under relevant environmental conditions

Crystalline proteins (δ endotoxins) are produced at the time of sporulation. They are exogenous metabolites of *Bacillus thuringiensis* with insecticide activity. These proteins are multicomponent proteins that are disaggregated on the single active components (Cry toxins) under favourable conditions. The production of this kind of proteins is the common characteristics of all *Bacillus thuringiensis* species. However, the actual proteins may vary from species to species and among different strains. The variations usually result in proteins selective to different kind of insects. *Bacillus thuringiensis* subsp. *israelensis* strain AM65-52 / VectoBac WG crystalline proteins are recognised to be relatively selective to: *dipterans.* These proteins are:

- Stable outside the micro organism
- Biologically active independently of the presence of the microorganism
- Intended to be applied at levels above background levels

Therefore, the data requirements and the corresponding risk assessment, according directive 91/414/EEC annex II part A point 7 (standard data requirements and assessment mandatory for chemical plant protections active substances), need to be fulfilled and performed for the *Bacillus thuringiensis* crystalline proteins.

No studies on the **route and rate of degradation in soil, mobility in soil and degradation in water and water sediment** of *Bacillus thuringiensis* subsp. *israelensis* strain AM65-52 crystalline proteins are available in the dossier. The assessment presented in the DAR is based on the summary of scientific publications submitted by the applicant in the dossier on other strains and or species of the *Bacillus thuringiensis*. The waiver based on the consideration of these proteins as pro toxins (that are only disaggregated in the toxic proteins in the gut of the insect) was not considered acceptable during the peer review, since there is evidence that the toxins can be released under naturally occurring environmental conditions outside the target organisms. Furthermore, it is common to many chemically synthetic pesticides, that the biologically active moiety is only formed through an enzymatic transformation within the target organism. This has never been considered as a justified reason to waive environmental exposure assessment, since it is regarded as part of the mechanism of action of the precursor pesticide. In the DAR it was stated that the persistence of the parasporal crystal proteins is expected to be short. However, no individual assessment of the publications was presented, so it is difficult to track back the statements and conclusions to the original scientific data. Worst case estimations of soil and water halve lives and soil adsorption would be needed to finalise the environment exposure assessment of these proteins. Potential **contamination of groundwater** is not addressed in the dossier. The experts at the PRAPeR meeting M3 identified a data gap for the groundwater exposure assessment for the crystal protein and conversion products that retain any insecticidal activity to be addressed. In the case of *Bacillus thuringiensis* subsp. *israelensis* strain AM65-52, only uses in greenhouse on potted plants are intended. Adequate management to prevent natural soil and groundwater contamination may be proposed in absence of further information.

Experimental information on the persistence of the crystal protein in **surface water** is not addressed in the DAR. The experts at PRAPeR meeting M3 agreed that no further data would be needed if the aquatic risk assessment could be finalised using an annual total dose PEC_{SW}. These have been

recalculated by EFSA and can be found in Appendix A. However this assessment only covers volatilization / deposition route, management measures tailored to local practice and legislation may need to be put in place to control the waste disposal of spent application solution and prevent accidental spillage entering sewers or surface water drains.

5. Ecotoxicology

It is noted that a data gap for the relevant metabolites was identified in the sections 2 and 4. Pending on this data gap, further information to address the ecotoxicological risk assessment for the aquatic organisms and soil organisms is required.

Some strain specific ecotoxicological studies to address the toxicity, infectiveness or pathogenicity of *Bacillus thuringiensis* subsp. *israelensis* strain AM65-52 on non-target organisms were available.

Regarding the aquatic organisms, some acute and chronic toxicity studies on fish and daphnids were available. A low risk from pathogenicity and infectiveness was indicated. Since the exposure to surface waters could not be excluded for the representative use in glasshouses (see fate and behaviour section) an aquatic risk assessment was carried out in the Addendum to chapter B.9. The risk for aquatic organisms was indicated as low by the TERs for the representative glasshouse use.

A toxicity study on earthworms was provided after the RMS got the dossier. However, no new studies can be taken into account in the peer-review according to Regulation 1095/2007.

Since environmental exposure from the representative use could be considered as negligible, the risk for birds, mammals, bees, non-target arthropods other than bees, terrestrial plants were considered as low.

The risk for soil micro organisms was addressed as low, by the available data.

The exposure of the organisms involved in the biological methods for sewage treatment plants could not be excluded, and no study or information was available, a data gap was identified.

6. Overview of the risk assessment of compounds listed in residue definitions triggering assessment of effects data for the environmental compartments

6.1. Soil

Compound (name and/or code)	**Persistence**	**Ecotoxicology**
Bacillus thuringiensis subsp. *israelensis* strain AM65-52 spores.	No data available. May persist months to years in soil	No data and required.
Bacillus thuringiensis subsp. *israelensis* strain AM65-52 crystalline proteins (δ endotoxins).	No data available.	No data and required.

6.2. Ground water

Compound (name and/or code)	**Mobility in soil**	**>0.1 µg/L 1m depth for the representative uses (at least one FOCUS scenario or relevant lysimeter)**	**Pesticidal activity**	**Toxicological relevance**	**Ecotoxicological activity**
Bacillus thuringiensis subsp. *israelensis* strain AM65-52 crystalline proteins (δ endotoxins).	No data available.	No data available. Data gap or proposal for management of soil residues to avoid groundwater contamination.	**No data. Not needed**	**Yes**	Low risk was indentified for the aquatic organisms.

6.3. Surface water and sediment

Compound (name and/or code)	**Ecotoxicology**

Bacillus thuringiensis subsp. *israelensis* strain AM65-52 spores.	Low risk was indentified for the aquatic organisms
Bacillus thuringiensis subsp. *israelensis* strain AM65-52 crystalline proteins (δ endotoxins).	No data and not required.

6.4. Air

Compound (name and/or code)	**Toxicology**
Bacillus thuringiensis subsp. *israelensis* strain AM65-52 spores.	No adverse effect after intratracheal instillation of $8x10^7$ cfu/rat.
Bacillus thuringiensis subsp. *israelensis* strain AM65-52 crystalline proteins (δ endotoxins).	No data available.

7. List of studies to be generated, still ongoing or available but not peer reviewed

This is a complete list of the data gaps identified during the peer review process, including those areas where a study may have been made available during the peer review process but not considered for procedural reasons (without prejudice to the provisions of Article 7 of Directive 91/414/EEC concerning information on potentially harmful effects).

- Validation of the method for protein content (parasporal body protein) (relevant for all representative uses evaluated; submission date proposed by the notifier: unknown; see section 1)
- Method for the unequivocal identification to strain level (relevant for all representative uses evaluated; submission date proposed by the notifier: unknown; see section 1)
- Demonstrate that the level of microbial contamination complies with international standards. The OECD issues paper (OECD, 2011) should be used as the reference. This should include analysis of batches for IU, CFU and protoxin levels (relevant for all representative uses evaluated; submission date proposed by the notifiers: unknown; see section 1).
- Validation of the methods of analysis for contaminating microorganisms (relevant for all representative uses evaluated; submission date proposed by the notifier: unknown; see section 1)
- Method to detect, isolate and enumerate the microorganism must be validated (relevant for all representative uses evaluated; submission date proposed by the notifier: unknown; see section 1)
- The biopotency method must be validated (relevant for all representative uses evaluated; submission date proposed by the notifier: unknown; see section 1)
- Optimal environmental growing conditions and the temperature range at which the organism grows is required for this strain (relevant for all representative uses evaluated; submission date proposed by the notifier: unknown; see section 1)
- Validation data for the methods of analysis for beta-exotoxin and enterotoxin (relevant for all representative uses evaluated; submission date proposed by the notifier: unknown; see section 1)
- Validated method for the CFU in the formulation (relevant for all representative uses evaluated; submission date proposed by the notifier: unknown; see section 1)
- Validation of the bridging of toxicological data between the formulation VectoBac 12 AS (detailed composition to be provided) and the representative formulation VectoBac WG (relevant for all representative uses evaluated; submission data proposed by the notifier: unknown; see section 2).
- Assessment of the production of toxins/secondary metabolites (e.g. enterotoxins, beta-exotoxin, cytolytic protein) after application and re-entry worker exposure is missing (relevant for all representative uses; submission data proposed by the notifier: unknown; see section 2).
- A data gap has been identified for scientific literature quoted by the applicant in its assessment of the fate and behaviour into the environment are not available in the dossiers submitted to RMS and EFSA (relevant for all representative uses evaluated; submission date proposed by the notifier: no date proposed; see section 4).
- No information has been provided in relation to potential interferences of *Bacillus thuringiensis* with the analytical systems for the control of the quality of drinking water provided for in Directive 98/83/EC (relevant for all representative uses evaluated; submission date proposed by the notifier: no date proposed; see section 4)

- No information has been provided on the potential transfer of genetic material from *Bacillus thuringiensis* to other organisms. (relevant for all representative uses evaluated; submission date proposed by the notifier: no date proposed; see section 2 and 4)

- A data gap has been identified to demonstrate that, under the conditions of use, *Bacillus thuringiensis* subsp. *israelensis* strain AM65-52 crystalline proteins (δ endotoxins) or any of their transformation products retaining insecticidal activity will not contaminate ground water above the regulatory limit of 0.1 µg /L. Further data on the persistence, transformation and mobility of δ endotoxins may be needed in order to fulfil this data gap. Alternatively a proposal for management measures to avoid the groundwater contamination after disposal residues of soil used in greenhouse (relevant for all representative uses evaluated; submission date proposed by the notifier: no submission date provided; see section 4)

- Pending on data gap identified in sections 2 and 4, further information to address the ecotoxicological risk assessment for the aquatic organisms and soil organisms. (relevant for all representative uses evaluated; submission date proposed by the notifier: no submission date provided; see section 5)

- The exposure of the organisms involved in the biological-methods for sewage treatment plants could not be excluded, (relevant for all representative uses evaluated; submission date proposed by the notifier: no submission date provided; see section 5)

8. Particular conditions proposed to *be taken in*to account to manage the risk(s) identified

- Aquatic environmental risk assessment available assumes exposure arising from representative uses in green house and emissions trough volatilisation and short range transport to surface water. However, management measures tailored to local practice and legislation may need to be put in place to control the waste disposal of spent application solution and prevent accidental spillage entering sewers or surface water drains. Potential for ground water contamination may also be addressed with adequate management and disposal of the soil/substrate used in the treated pots (see section 4).

9. Concerns

9.1. Issues that could not be finalised

An issue is listed as an issue that could not be finalised where there is not enough information available to perform an assessment, even at the lowest tier level, for the representative uses in line with the Uniform Principles of Annex VI to Directive 91/414/EEC and where the issue is of such importance that it could, when finalised, become a concern (which would also be listed as a critical area of concern if it is of relevance to all representative uses).

1. Pending on their potential exposure to toxins produced after application, the risk assessment cannot be finalised for the re-entry workers.

2. No information has been provided in relation to potential interferences of *Bacillus thuringiensis* with the analytical systems for the control of the quality of drinking water provided for in Directive 98/83/EC.

3. Assessment of potential transfer of genetic material cannot be finalised.

4. The risk for the organisms involved in the biological methods for sewage treatment plants.

9.2. Critical areas of concern

An issue is listed as a critical area of concern where there is enough information available to perform an assessment for the representative uses in line with the Uniform Principles of Annex VI to Directive 91/414/EEC, and where this assessment does not permit to conclude that for at least one of the representative uses it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater or any unacceptable influence on the environment.

An issue is also listed as a critical area of concern where the assessment at a higher tier level could not be finalised due to a lack of information, and where the assessment performed at the lower tier level does not permit to conclude that for at least one of the representative uses it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater or any unacceptable influence on the environment.

- None.

9.3. Overview of the concerns identified for each representative use considered

(If a particular condition proposed to be taken into account to manage an identified risk, as listed in section 8, has been evaluated as being effective, then 'risk identified' is not indicated in this table.)

Representative use		**Ornamentals (G)**
Operator risk	Risk identified	
	Assessment not finalised	
Worker risk	Risk identified	
	Assessment not finalised	X[1]
Bystander risk	Risk identified	
	Assessment not finalised	
Consumer risk	Risk identified	
	Assessment not finalised	
Risk to wild non target terrestrial vertebrates	Risk identified	
	Assessment not finalised	
Risk to wild non target terrestrial organisms other than vertebrates	Risk identified	
	Assessment not finalised	
Risk to aquatic organisms	Risk identified	
	Assessment not finalised	X[4]

Groundwater exposure active substance	Legal parametric value breached	
	Assessment not finalised	X[2]
Groundwater exposure metabolites	Legal parametric value breached	
	Parametric value of 10µg/L[(a)] breached	
	Assessment not finalised	
Comments/Remarks		

The superscript numbers in this table relate to the numbered points indicated in sections 9.1 and 9.2. Where there is no superscript number see sections 2 to 6 for further information.
(a): Value for non-relevant metabolites prescribed in SANCO/221/2000-rev 10-final, European Commission, 2003.

REFERENCES

Italy, 2007. Draft Assessment Report (DAR) on the active substance *Bacillus thuringiensis israelensis* AM65-52 prepared by the rapporteur Member State Italy in the framework of Directive 91/414/EEC, November 2007

Italy, 2012. Final Addendum to Draft Assessment Report on *Bacillus thuringiensis israelensis* AM65-52 ., compiled by EFSA, October 2012.

EFSA (European Food Safety Authority), 2012. Peer Review Report to the conclusion regarding the peer review of the pesticide risk assessment of the active substance *Bacillus thuringiensis israelensis* AM65-52

EFSA, 2007. Introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA. Opinion of the Scientific Committee. The EFSA Journal (2007) 587, 1-16.

EFSA (European Food Safety Authority), 2009. Guidance Document on Risk Assessment for Birds and Mammals on request of EFSA. EFSA Journal 2009; 7(12):1438.

EFSA, 2008. Scientific Opinion of the Panel on Biological Hazards. The maintenance of the list of QPS microorganisms intentionally added to food or feed. The EFSA Journal (2008) 923, 1-48.

European Commission, 2008. Review Report for the active substance *Bacillus thuringiensis israelensis* AM65-52 finalised in the Standing Committee on the Food Chain and Animal Health at its meeting on 11 July 2008 in view of the inclusion of *Bacillus thuringiensis israelensis* AM65-52 in Annex I of Directive 91/414/EEC SANCO/1540/08-rev.3, 3 June 2008.

FOCUS (2008). "Pesticides in Air: Considerations for Exposure Assessment". Report of the FOCUS Working Group on Pesticides in Air, EC Document Reference SANCO/10553/2006 Rev 2 June 2008.

APPENDICES

APPENDIX A – LIST OF END POINTS FOR THE ACTIVE SUBSTANCE AND THE REPRESENTATIVE FORMULATION

Chapter 1 Identity, Biological properties, Details of Uses, Further Information

Active micro-organism:	*Bacillus thuringiensis* subspecies *israelensis*, (Serotype H-14), Strain AM65-52.
Function:	Insecticide
Country to which application is made:	Italy.

Identity of the Microbial Pest control Agent (MPCA) (OECD data point IIM 1)	
Name of the organism:	*Bacillus thuringiensis* subspecies *israelensis*, (Serotype H-14), Strain AM65-52.
Taxonomy:	*Bacillus thuringiensis* subsp *israelensis,* seropytpe H-14, strain AM 65-52 is a facultative anaerobic, gram-positive bacterium that forms characteristic protein inclusions adjacent to the endospore. The basic phenotypic toxin is the subspecies, identified by flagella (H) serotype.
Species, subspecies, strain:	*Bacillus thuringiensis* subspecies *israelensis*, (Serotype H-14), Strain AM65-52.
Identification/detection:	Open for validated strain specific method.
Culture collection:	American Type Culture Collection no. ATCC - 1276
Minimum and maximum concentration of the MPCA used for manufacturing of the formulated product:	Bti (Strain AM65-52) technical grade contains high and low limits of 20% and 8%, respectively of *Bacillus thuringiensis* subsp. *israelensis* solids, spores and insecticidal toxins.
Identity and content of relevant impurities, additives, contaminating organisms in the technical grade of MPCA:	Open for contaminating microorganisms.
Is the MPCA genetically modified if so provide type of modification:	No

Biological properties of the micro-organism (OECD data point IIM 2)	
Origin and natural occurrence:	*Bacillus thuringiensis* subsp *israelensis* is a naturally occurring micro-organism first described by de Barjac (1978).
Background level:	*Bacillus thuringiensis* subsp *israelensis* occurs ubiquitously at low levels in soil and on plants and insects.
Target organism (s)	Larvae of fungus gnats (*Diptera sciaridae*).
Mode of action:	The mode of action of Bti (Strain AM65-52) results from toxic proteins contained in parasporal crystals. The crystals are taken up via ingestion and under the alkali conditions present in the larvae gut the crystal dissolves releasing the active protein delta endotoxins that induce disintegration of the larvae gut epithelium and consequent death of the larvae.
Host specificity:	Bti (Strain AM65-52) exhibits specific toxicity to *Dipteran* insects upon ingestion.
Life cycle:	The life-cycle of Bti (Strain AM65-52) follows the characteristic process of spore formation (sporulation) typical of *Bacillus* cultures, with the exception that insect toxin containing parasporal bodies are formed during sporulation.
Infectivity, dispersal and colonisation ability:	See sections 2, 4, 5.
Relationships to known plant animal and human pathogens:	*Bacillus thuringiensis* is related to known human pathogens *Bacillus anthracis* and *Bacillus cereus* that form toxic metabolites.

Genetic stability:	Open; see sections 2 and 4.
Information on the production of relevant metabolites (especially toxins):	The mode of action of Bti (Strain AM65-52) results from toxic proteins contained in parasporal crystals. There are no other active metabolites and degradation products that are known to contribute to the toxicity of Bti (Strain AM65-52). The presence of beta-exotoxins and enterotoxins which may be produced by other *Bacillus thuringiensis* subspecies is monitored and controlled during the manufacturing process and do not occur in Bti Strain AM65-52. However it is not known if this strain can produce the enterotoxin in the human gut.
Resistance/sensitivity to antibiotics/anti-microbial agents used in human or veterinary medicine:	Bti (Strain AM65-52) is susceptible to gentamicin, kanamycin, erythromycin, clindamycin, vancomycin, chloramphenicol and trimethoprim/sulphamethoxazole, but is resistant to penicillin, ampicillin and cephalothrin.

Classification and proposed labelling	
With regard to physical/chemical data:	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

Summary of intended uses

Crop and /or situation (a)	Member state or Country	Product name	F G or I (b)	Pest or Group of pests controlled (c)	Formulation*		Application**				Application rate per treatment			PHI (days) (l)	Remarks (m)
					Type (d-f)	Conc. of as (i)	Method Kind (f-h)	Growth stage & season (j)	Number min max	Interval between applications (min)	kg ai/hl g ai/m^2 min max	water l/ha min max	kg ai/ha min max		
Ornamental (pot plants)	Indoor/all EU regions	VectoBac (Gnatrol)	I	Sciarid flies (fungus gnat)	WG	37.4%	Spray	–	1-3	8-10 days	3 Kg/hl	1000 L/ha or 0.1 L/m^2	3 g ai/m^2	NA	–

(a) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (*e.g.* fumigation of a structure)
(b) Outdoor or field use (F), glasshouse application (G) or indoor application (I)
(c) *e.g.* biting and suckling insects, soil born insects, foliar fungi, weeds
(d) *e.g.* wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
(e) GCPF Codes - GIFAP Technical Monograph No 2, 1989
(f) All abbreviations used must be explained
(g) Method, *e.g.* high volume spraying, low volume spraying, spreading, dusting, drench
(h) Kind, *e.g.* overall, broadcast, aerial spraying, row, individual plant, between the plant - type of equipment used must be indicated
(i) Cfu=colony forming units and g/kg or g/l
(j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(k) Indicate the minimum and maximum number of application possible under practical conditions of use
(l) PHI - minimum pre-harvest interval
(m) Remarks may include: Extent of use/economic importance/restrictions

NA = Not applicable. Product is applied to ornamental crops only and a PHI is not appropriate for this use.
* Formulation contains 37.4% by weight *Bacillus thuringiensis*, subsp. *israelensis*, strain AM 65-52 fermentation solids and solubles.
** The product should be applied by spray over the pot plants at a volume of 1000 litres/ha, or 0.1 litre/m^2 on a moving platform. Sufficient spray should be applied to drench the substrate.

Further information

Production control	Microbiological and biochemical tests are performed to determine the content of impurities, biopotency and absence of toxic metabolites
Proposal for classification and labelling	Warning phrase: "Microorganisms may have the potential to provoke sensitising reactions."

Chapter 2 Analytical Methods

Analytical methods for the micro-organism

Manufactured micro-organism (principle of the method):	Open
Impurities and contaminating micro-organisms in manufactured material (principle of method:	Open for methods for toxins and contaminating microorganisms.
Microbial Pest Control Product (principle of the method):	Open

Analytical methods for residues (viable and non-viable) in exposed compartments and organisms OECD data point IIM 4.5)	
Residues of the active micro-organism (principle of the method):	Subject to the outcome of the environmental assessment.
Residues of relevant metabolites (principle of the method):	Not applicable.

Chapter 3 Effects on Human Health

Medical data, surveillance and observations:	No evidence of adverse health effect among manufacturing plant personnel exposed to *Bacillus thuringiensis israelensis (Bti).* Few incidences of adverse effects associated with *Bacillus thuringiensis* exposure are reported in the literature. No major effects were observed in populations exposed to aerial spraying of *Bacillus thuringiensis kurstaki.*

Sensitisation:	Human patch testing with *Bacillus thuringiensis kurstaki* revealed no cases of contact hypersensitivity. Without evidence of allergic symptoms or infectious diseases, positive IgE tests were found among farm workers highly exposed to *Bacillus thuringiensis israelensis*. *Bti* was shown to be skin sensitiser in a Buehler test with guinea pigs. Considering that all microbials should be regarded as potential sensitizers, the agreed warning phrase is "Micro-organisms may have the potential to provoke sensitising reactions".

Toxicity

after acute oral exposure:	- rat LD_{50} > 5000 mg/kg bw (~5.5 x 10^{11} CFU/kg bw) -10^8 CFU/animal had no adverse effects on rats - 2.0x10^9 CFU/animal did not induce adverse effects in rats.
after acute inhalation exposure:	- Intratracheal instillation of 10^8 CFU/rat resulted in no mortality, the observed clinical signs and lung lesions were attributed to the presence of foreign material rather than to an infective process - Intratracheal instillation of 8x10^7 CFU/rat resulted in no adverse effects. - after aerosol exposure of rats (whole body; 2.84 mg/L, i.e. ~3.2x10^8 CFU/L), no deaths occurred and no clinical signs were observed after day 1.
after acute intravenous exposure:	10^7 CFU/rat induced no treatment related toxic effects.
after single intraperitoneal exposure:	No clinical signs of toxicity in mice at doses of 10^6, 10^7 or 10^8 CFU/animal, no post-mortem examinations.

Infectivity

after acute oral exposure:	Clearance from organs by day 8, clearance from faeces at day 22.
after acute inhalation exposure:	- total clearance was incomplete after intratracheal instillation (day 50 after instillation) - no signs of infectious disease
after intravenous exposure:	Uncomplete clearance at day 50 in lungs, spleen, liver, lymph nodes and kidneys.
after intraperitoneal exposure:	Clearance not investigated.

Pathogenicity

after acute oral exposure:	No indication of pathogenic effects.
after acute inhalation exposure:	No indication of pathogenic effects.
after intravenous exposure:	No indication of pathogenic effects.
after intraperitoneal exposure:	No indication of pathogenic effects.

Genotoxicity:	Standard mutagenicity and genotoxicity assays are not appropriate for

	many living microorganisms. Potential toxins might need to be further investigated for their genotoxic properties.
Cell culture study:	This is not applicable to *Bacillus thuringiensis* which has not been shown to replicate in warm-blooded organisms.

Information on short-term toxicity and pathogenicity:	- 90-day dog (oral gavage): NOAEL $5x10^6$ spores/animal. No treatment-related adverse effect was observed (supplementary information) - 14-day rat (4h/d, nose-only): NOAEL $1.84x10^6$ spores/L. No treatment-related adverse effect was observed (supplementary information)
Dermal toxicity:	Dermal LD_{50} in rabbits >5000 mg/kg bw VectoBac Technical Powder). No dermal reaction or adverse effect after application of *Bti* to abraded rabbit skin, or after ocular instillation. *Bti* induced a mild irritation of the skin and the eye in albino rabbits.
Specific-toxicity, pathogenicity and infectivity:	In a range of toxicological studies with *Bacillus thuringiensis* serotype H-14, experimental infection of mice, rats, guinea pigs and rabbits was attempted by various routes. Doses of 10^7 to 10^8 CFU/animal in acute studies, up to 10^{12} CFU/animal in subacute studies, resulted in no adverse toxic effects and no signs of virulence or indication of anaphylaxis. (supplementary data)

Reference values

AOEL	Not applicable based on the lack of pathogenicity and infectivity in the available data.
ADI	Not applicable based on the lack of pathogenicity and infectivity in the available data.

Exposure scenarios

Application method	Indoor application on ornamentals
Operators	Estimation of exposure not required because the product does not raise significant toxicological concerns.
Workers	Estimation of exposure not required because the product does not raise significant toxicological concerns. Pending on their exposure to toxins potentially produced after application, the risk assessment cannot be finalised.
Bystanders	Estimation of exposure not required because the product does not raise significant toxicological concerns.

Chapter 4 Residues

Bti (Strain AM65-52) is recommended for the control of fungus gnats (*Sciaridae*) in ornamental plant cultivation. Bti (Strain AM65-52) is not infective and is not toxic or pathogenic to mammalian species. Data on potential residues of Bti (Strain AM65-52) in food or feedstuffs are therefore not relevant and it is not considered necessary to propose an Acceptable Daily Intake (ADI), calculate the potential exposure of consumers, or propose a Maximum Residue Level (MRL) for this MCPA.

Residues in or on treated products, food and feed

Viable residues	Not applicable
Non-viable residues	Not applicable

Chapter 5 Fate and Behaviour in the Environment

Persistence and multiplication in soil	No data on the persistence and multiplication of *Bacillus thuringiensis* subsp. *israelensis* strain AM65-52 in soil is available in the dossier. *Bacillus thuringiensis* occurs naturally and ubiquitously in the environment. It is a common component of the soil micro-biota and has been isolated from most terrestrial habitat. Available information indicates that *Bacillus thuringiensis* spores may persist from months to years in soil under natural field conditions. The low potential for spore germination, growth and re-sporulation in soils is expected to restrict population growth. Assuming no degradation, following three applications of 30 kg MPCA/ha the initial PECsoil values for VectoBac WG after the last application are: PEC soil: 39.1 mg /kg dry weight soil 1.9×10^9 CFU /kg dry weight soil However, it should be noted that the intended use is on potted plants in greenhouses. Risk management measures are expected to be put in place to avoid the release of contaminated soil into the environment.
Persistence and multiplication in water an air	No data on the persistence and multiplication of *Bacillus thuringiensis* subsp. i*sraelens* strain AM65-52 in aquatic environment is available in the dossier. According available information, *B. thuringiensis* is not regarded as an autochthonous inhabitant of aquatic environments and does not find optimal conditions for growth. Therefore, proliferation is not likely to occur. PEC_{SW} in terms of viable spores (cfu) of active substance: Application rate: $3 \times 3 \times 10^{13}$ cfu/ha Amount reaching the soil: 9×10^{13} cfu/ha Relevant value for spray drift (FOCUS air for indoor uses[Focus (2008)]): 0.05 %, resulting in 4.5×10^{10} cfu Amount reaching the water: 4.5×10^{10} cfu Initial PECsw assuming a water volume of 30000 L: **1.5×10^4 cfu/l** PEC_{SW} in terms of crystal protein Application rate: 3 x 30 kg/ha Amount reaching the soil: 90 kg/ha Relevant value for spray drift (FOCUS air for indoor uses[Focus (2008)]): 0.05 %, resulting in g Amount reaching the water: 45 g Initial PECsw assuming a water volume of 30000 L: **15 µg/l** This assessment covers volatilization / deposition route, management measures tailored to local practice and

	legislation may need to be put in place to control the waste disposal of spent application solution.
Persistence and multiplication in air	No data available. As for other microbial spores, degradation due to solar radiation may be expected.
Mobility	**Spores**: According available information, it has been concluded that movement of Bt spores through the soil by leaching is unlikely to occur. **Crystalline proteins (δ-endotoxins):** data gap identified to address potential ground water contamination. However, in the case of *Bacillus thuringiensis* subsp. *israelensis* strain AM65-52 only uses in greenhouse on potted plants are intended. Adequate management to prevent groundwater contamination may be put in place in absence of further information.

Chapter 6 Effects on Non-target Species

Effects on birds				
Application rate (kg MPCA/ha)	Crop	Category (eg insectivorous bird)	Time-scale	Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects)
3 g a.s./m^2	Ornamental pot plants	Mallard duck	Short-term	LC_{50} >3077 mg/kg per day (equivalent to a daily dose of 716 mg/kg bw/day); LC_{50} > 6.2 x 10^{11} CFU/g
3 g a.s./m^2	Ornamental pot plants	Northern bobwhite	Short-term	LC_{50} >3077 mg/kg per day (equivalent to a daily dose of 1874 mg/kg bw/day); LC_{50} > 6.2 x 10^{11} CFU/g

The risk assessment was not needed.

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 a.s./L
Lepomis macrochirus	*Bti*	Acute 96-hours	96h LC_{50} >600 mg a.s./L
Oncorhynchus mykiss	Vectobac technical	Chronic 32-day	NOEC - 1.1x 10^{10} CFU/L aqueous exposure; NOEC – 0.055 g/L
Lepomis macrochirus	Vectobac technical	Chronic 30-day	NOEC - 1.2 x 10^{10} CFU/L aqueous exposure; NOEC – 0.06 g/L.
Cyprinodon variegatus	Vectobac technical	Chronic 30-day	NOEC - 1.3 x 10^{10} CFU/L aqueous exposure; NOEC – 0.065 g/L
Laboratory tests – invertebrate species			
Daphnia magna	Vectobac technical	Chronic 10-day	10-day LC_{50} - >50 mg a.s./L;
Daphnia magna	Vectobac technical	Chronic 21-day	NOEC = 5 mg a.s./L, NOEC = 1 x 10^{9} CFU/L
Grass shrimp (*Palaemonetes vulgaris)*	Vectobac technical	Chronic 31-day	NOEC = 2.0 x 10^{10} CFU/g dietary concentration; NOEC = 0.1 g MPCA/L
Mayfly nymphs (*Hexagenia* sp)	Vectobac technical	Chronic 18-day	NOEC = 2.0 x 10^{10} CFU/L aqueous concentration ; NOEC = 0.1 g MPCA/L
Amphiascus minutus	Vectobac technical	Chronic 10-day	10-day LC_{50} - >50 mg a.s./L; 10-day LC_{50} - > 1x10^{10} CFU /L NOEC - 50 mg a.s./L; NOEC - 1x10^{10} CFU/L
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* var. *israelensis*, Strain 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* (Strain 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* (Strain AM65-52) and therefore no appropriate digestive enzymes to enable the release of the active protein delta endotoxins.

Aquatic organisms	End-point	PECsw	TER
Amphiascus minutus	**10-day LC_{50} - > $1x10^{10}$ CFU /L**	**1.5 x 10^6 cfu/l**	9999.9

Effects on bees	LD_{50}/24h (µg product/bee)	LD_{50}/24h (CFU product/bee)	LD_{50}/48h (µg product/bee)	LD_{50}/48h (µg product/bee)
Oral toxicity test	> 108.04	> $2.16x10^5$	> 108.04	> $2.16x10^5$
Contact toxicity test	> 100	> $2x10^5$	> 100	> $2x10^5$

The risk assessment was not needed.

Effects on other arthropods species
No studies have been conducted on other arthropod species. According to the Applicant this has been considered not relevant owing to the use in pot cultures However, it has been considered that the ornamental plants are not often grown in pots but in soil under greenhouse conditions. Moreover, during spring- summer- and autumn-time frequently the greenhouses are opened for most of the time. However, it has to be considered the use of the product on pot soil.

The risk assessment was not needed.

Effects on earthworms		
Product	Species	Not specified
Data available were submitted after the dossier and they were not considered according to the procedure		

Effects on non-target soil micro-organisms
A study on the effects on non-target soil micro-organisms has been provided by the Applicant. 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 ICPs from Bti against against the above bacteria were observed.

Additional studies	
Nitrogen mineralisation:	No additional studies were conducted and not needed.
Carbon mineralisation:	No additional studies were conducted and not needed.

APPENDIX B – USED COMPOUND CODE(S)

Code/Trivial name*	Chemical name	Structural formula

ABBREVIATIONS

1/n	slope of Freundlich isotherm
λ	wavelength
ε	decadic molar extinction coefficient
°C	degree Celsius (centigrade)
µg	microgram
µm	micrometer (micron)
a.s.	active substance
AChE	acetylcholinesterase
ADE	actual dermal exposure
ADI	acceptable daily intake
AF	assessment factor
AOEL	acceptable operator exposure level
AP	alkaline phosphatase
AR	applied radioactivity
ARfD	acute reference dose
AST	aspartate aminotransferase (SGOT)
AV	avoidance factor
BCF	bioconcentration factor
BUN	blood urea nitrogen
bw	body weight
CAS	Chemical Abstracts Service
CFU	colony forming units
ChE	cholinesterase
CI	confidence interval
CIPAC	Collaborative International Pesticides Analytical Council Limited
CL	confidence limits
cm	centimetre
d	day
DAA	days after application
DAR	draft assessment report
DAT	days after treatment
DM	dry matter
DT_{50}	period required for 50 percent disappearance (define method of estimation)
DT_{90}	period required for 90 percent disappearance (define method of estimation)
dw	dry weight
EbC_{50}	effective concentration (biomass)
EC_{50}	effective concentration
ECHA	European Chemical Agency
EEC	European Economic Community
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINCS	European List of New Chemical Substances
EMDI	estimated maximum daily intake
ER_{50}	emergence rate/effective rate, median
ErC_{50}	effective concentration (growth rate)
EU	European Union
EUROPOEM	European Predictive Operator Exposure Model
f(twa)	time weighted average factor
FAO	Food and Agriculture Organisation of the United Nations
fd	feed
FIR	Food intake rate
FOB	functional observation battery
FOCUS	Forum for the Co-ordination of Pesticide Fate Models and their Use
g	gram

GAP	good agricultural practice
GC	gas chromatography
GCPF	Global Crop Protection Federation (formerly known as GIFAP)
GGT	gamma glutamyl transferase
GM	geometric mean
GS	growth stage
GSH	glutathion
h	hour(s)
ha	hectare
Hb	haemoglobin
Hct	haematocrit
hL	hectolitre
HPLC	high pressure liquid chromatography or high performance liquid chromatography
HPLC-MS	high pressure liquid chromatography – mass spectrometry
HQ	hazard quotient
IEDI	international estimated daily intake
IESTI	international estimated short-term intake
ISO	International Organisation for Standardisation
IU	International Unit
IUPAC	International Union of Pure and Applied Chemistry
JMPR	Joint Meeting on the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues (Joint Meeting on Pesticide Residues)
K_{doc}	organic carbon linear adsorption coefficient
kg	kilogram
K_{Foc}	Freundlich organic carbon adsorption coefficient
L	litre
LC	liquid chromatography
LC_{50}	lethal concentration, median
LC-MS	liquid chromatography-mass spectrometry
LC-MS-MS	liquid chromatography with tandem mass spectrometry
LD_{50}	lethal dose, median; dosis letalis media
LDH	lactate dehydrogenase
LOAEL	lowest observable adverse effect level
LOD	limit of detection
LOQ	limit of quantification (determination)
m	metre
M/L	mixing and loading
MAF	multiple application factor
MATC	maximum allowable toxicant concentration
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
mg	milligram
mL	millilitre
mm	millimetre
mN	milli-newton
MPCA	Microbial pest control agent
MRL	maximum residue limit or level
MS	mass spectrometry
MSDS	material safety data sheet
MTD	maximum tolerated dose
MWHC	maximum water holding capacity
NESTI	national estimated short-term intake

ng	nanogram
NOAEC	no observed adverse effect concentration
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
NOEL	no observed effect level
OD	oil dispersion
OECD	Organisation for Economic Co-operation and Development
OM	organic matter content
Pa	pascal
PD	proportion of different food types
PEC	predicted environmental concentration
PEC_{air}	predicted environmental concentration in air
PEC_{gw}	predicted environmental concentration in ground water
PEC_{sed}	predicted environmental concentration in sediment
PEC_{soil}	predicted environmental concentration in soil
PEC_{sw}	predicted environmental concentration in surface water
pH	pH-value
PHED	pesticide handler's exposure data
PHI	pre-harvest interval
PIE	potential inhalation exposure
pK_a	negative logarithm (to the base 10) of the dissociation constant
P_{ow}	partition coefficient between *n*-octanol and water
PPE	personal protective equipment
ppm	parts per million (10^{-6})
ppp	plant protection product
PT	proportion of diet obtained in the treated area
PTT	partial thromboplastin time
QSAR	quantitative structure-activity relationship
r^2	coefficient of determination
REACH	Registration, Evaluation, Authorisation of CHemicals
RPE	respiratory protective equipment
RUD	residue per unit dose
SC	suspension concentrate
SD	standard deviation
SFO	single first-order
SSD	species sensitivity distribution
STMR	supervised trials median residue
$t_{1/2}$	half-life (define method of estimation)
TER	toxicity exposure ratio
TER_A	toxicity exposure ratio for acute exposure
TER_{LT}	toxicity exposure ratio following chronic exposure
TER_{ST}	toxicity exposure ratio following repeated exposure
TK	technical concentrate
TLV	threshold limit value
TMDI	theoretical maximum daily intake
TRR	total radioactive residue
TSH	thyroid stimulating hormone (thyrotropin)
TWA	time weighted average
UDS	unscheduled DNA synthesis
UV	ultraviolet
W/S	water/sediment
w/v	weight per volume
w/w	weight per weight
WBC	white blood cell
WG	water dispersible granule

WHO	World Health Organisation
wk	week
yr	year