CONCLUSION ON PESTICIDE PEER REVIEW

Conclusion on the peer review of the pesticide risk assessment of the active substance *Bacillus thuringiensis* subsp. *kurstaki (strains ABTS 351, PB 54, SA 11, SA 12, EG 2348)*[1]

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SUMMARY

Bacillus thuringiensis subsp. kurstaki (strains ABTS 351, PB 54, SA 11, SA 12, EG 2348) 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 subsp. kurstaki (strains ABTS 351, PB 54, SA 11, SA 12, EG 2348) 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[5], in accordance with Commission Implementing Regulation (EU) No 540/2011[6], as amended by Commission Implementing Regulation (EU) No 541/2011[7]. In accordance with Article 25a of the Regulation, as amended by Commission Regulation (EU) No 114/2010[8], 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.

Denmark being the designated rapporteur Member State submitted a DAR on *Bacillus thuringiensis subsp. kurstaki* (strain ABTS 351) a DAR on *Bacillus thuringiensis subsp. kurstaki* (strain PB 54) and a DAR on *Bacillus thuringiensis subsp. kurstaki* (strain SA 11, SA 12, EG 2348) in accordance with the provisions of Article 22(1) of the Regulation, which were received by the EFSA on 4 February 2008. The peer review was initiated on 19 April 2008 by dispatching the DAR on *Bacillus thuringiensis subsp. kurstaki* (strain PB 54) for consultation of the notifier (Probelte S.A.) and the DARs on *Bacillus thuringiensis subsp. kurstaki* (strains ABTS 351, SA 11, SA 12, EG 2348) on 22 April 2008 for consultation of the notifiers (Mitsui AgriScience International S.A./B.V. and Valent BioScience). Subsequently the DARs were dispatched to the Member States on 11 June 2008. Following consideration of the comments received on the DARs, it was concluded that EFSA should

[1] On request from the European Commission, Question No EFSA-Q-2009-00248 adopted on 16 December 2011.
[2] Correspondence: pesticides.peerreview@efsa.europa.eu
[3] OJ L 379, 24.12.2004, p.13
[4] OJ L 246, 21.9.2007, p.19
[5] OJ L 309, 24.11.2009, p.1
[6] OJ L 153, 11.6.2011, p.1
[7] OJ L 153, 11.6.2011, p.187
[8] OJ L 37, 10.2.2010, p.12

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conduct a peer review and deliver its conclusions on *Bacillus thuringiensis subsp. kurstaki* (strains ABTS 351, PB 54, SA 11, SA 12, EG 2348 and deliver its conclusions on *Bacillus thuringiensis subsp. kurstaki* (strains ABTS 351, PB 54, SA 11, SA 12, EG 2348

The conclusions laid down in this report were reached on the basis of the evaluation of the representative uses of as an insecticide grapes, tomatoes and cabbage 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 toxin formation, contaminating microorganisms and methods of analysis both for identification and quantification.

In the area of mammalian toxicology the risk assessment cannot be finalised for operators, workers and bystanders, due to the lack of data on toxin formation. Three data gaps were identified: the test material used in the toxicology studies for the two formulations with the strain SA-11 has to be checked for its compliance to the formulations containing SA-12 and EG-2348, the acute inhalation toxicity of the WP product containing strain EG-2348 has to be addressed, and the acute oral and inhalative toxicity of the WP product with PB-54 has also to be addressed.

For residues the risk assessment cannot be finalised as the possible formation of enterotoxins cannot be excluded.

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 or assessment to demonstrate that this transfer does not occur or in case of occurring will not lead to unacceptable effects on human and animal health, and on the environment has to be provided.

No studies on persistence and multiplication in soil and aquatic environment of *Bacillus thuringiensis* subsp. *kurstaki strains* PB54, ABTS-351, SA-11, SA-12 and EG-2348 are available. Available data suggest that spores may remain in soil from months to years under field conditions. An estimation of background levels of spores and the time needed to recover after application of *Bacillus thuringiensis* subsp. *kurstaki* is currently not available.

Crystalline proteins (δ endotoxins) are produced at the time of sporulation. They are exogenous metabolites of *Bacillus thuringiensis* with insecticide activity. 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. *kurstaki strains* PB54, ABTS-351, SA-11, SA-12 and EG-2348 crystalline proteins are available. Worst case estimations of soil and water half-lives and soil adsorption would be needed to finalise the environment exposure assessment of these proteins. During the peer review a data gap was identified for the groundwater exposure assessment for the crystal protein and conversion products that retain any insecticidal activity.

Data gaps were identified for strains PB-54 SA-12: 1) to provide specific studies on *Daphnia magna*. due to the uncertainties on the extrapolation from other strains or alternatively a robust argumentation would be needed to support the extrapolation from other *Bacillus thuringiensis* subsp. *kurstaki* strains data.; 2) to provide a justification whether or not extrapolation on arthropod effect data from other strains is appropriate. A data gap was also identified to provide the papers mentioned in the addendum on the acute toxicity of *Bacillus thuringiensis kurstaki* to Lepidoptera, used to address the off-crop risk to non-target Lepidoptera population.

KEY WORDS

Bacillus thuringiensis kurstaki strains ABTS-351, PB54, SA-11, SA-12 and EG 2348, peer review, risk assessment, pesticide, insecticide.

TABLE OF CONTENTS

BACKGROUND

Bacillus thuringiensis subsp. kurstaki (strains ABTS 351, PB 54, SA 11, SA 12, EG 2348) is one of the 295 substances of the fourth stage of the review programme covered by Commission Regulation (EC) No 2229/2004[9], as amended by Commission Regulation (EC) No 1095/2007[10].

Bacillus thuringiensis subsp. kurstaki (strains ABTS 351, PB 54, SA 11, SA 12, EG 2348) was included in Annex I to Directive 91/414/EEC on 18 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[11], in accordance with Commission Implementing Regulation (EU) No 540/2011[12], as amended by Commission Implementing Regulation (EU) No 541/2011[13]. In accordance with Article 25a of the Regulation, as amended by Commission Regulation (EU) No 114/2010[14] the European Food Safety Authority (EFSA) is required to deliver by 31 December 2012 its view on the draft review reports submitted by the European Commission in accordance with Article 25(1) of the Regulation (European Commission, 2008a, b, c). 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.

Denmark being the designated rapporteur Member State submitted a DAR on *Bacillus thuringiensis subsp. kurstaki* (strain ABTS 351) a DAR on *Bacillus thuringiensis subsp. kurstaki* (strain PB 54) and a DAR on *Bacillus thuringiensis subsp. kurstaki* (strain SA 11, SA 12, EG 2348) in accordance with the provisions of Article 22(1) of the Regulation, which were received by the EFSA on 4 February 2008. The peer review was initiated on 19 April by dispatching the DAR on *Bacillus thuringiensis subsp. kurstaki* (strain PB 54) for consultation of the notifier (Probelte S.A.) and the DARs on *Bacillus thuringiensis subsp. kurstaki* (strains ABTS 351, SA 11, SA 12, EG 2348) on 22 April 2008 for consultation of the notifiers (Mitsui AgriScience Internationla S.A./B.V. and Valent BioScience). Subsequently the DARs were dispatched to the Member States on 11 June 2008. 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.

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, including those issues to be considered in consultation with Member State expert, and additional information to be submitted by the notifiers, were compiled by the EFSA in the format of an 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/December 2011.

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 grapes, tomatoes and cabbage, as proposed by the notifiers. 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

[9] OJ L 379, 24.12.2004, p.13
[10] OJ L 246, 21.9.2007, p.19
[11] OJ L 309, 24.11.2009, p.1
[12] OJ L 153, 11.6.2011, p.1
[13] OJ L 153, 11.6.2011, p.187
[14] OJ L 37, 10.2.2010, p.12

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, 2011) 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 (30 April 2009)
- the Evaluation Table (12 December 2011)
- the reports 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 2011) containing all individually submitted addenda (Denmark, 2011)) and the Peer Review Report, both documents are considered respectively as background documents A and B to this conclusion.

THE IDENTITY OF THE MICRO-ORGANISM AND THE PROPERTIES OF THE FORMULATED PRODUCT

Bacillus thuringiensis subsp. kurstaki strain ABTS-351 was isolated from diseased black larvae of the Pink Bollworm, Pectinophora gossypiella.

The representative formulation evaluated is 'Dipel WG' containing nominally 1.17x 10^{13} CFU/kg

The representative use evaluated is on outdoor cabbages as a foliar spray to control cabbage moth. Full details of the GAP can be found in the list of end points in Appendix A-1.

Bacillus thuringiensis subsp. kurstaki strain PB54 was isolated from soil in Spain.

The representative formulation for evaluation is 'Belthirul' containing nominally 5.09 x 10^{12} CFU/kg

The representative use evaluated is on tomatoes as a foliar spray to control tomato fruit worm. Full details of the GAP can be found in the list of end points in Appendix A-2.

The *Bacillus thuringiensis subsp. kurstaki* strains SA-11, SA-12 and EG 2348 were all isolated from insects.

The representative formulations for evaluation are 'Javelin WG', 'Delfin WG', 'CoStar WG', 'Deliver WG', 'Condor WP' and 'Rapax WP'. All formulations were stated to be identical in their composition by the RMS and contain nominally 4.85 x10^{13} CFU/kg. However, because of comments received by section 2 this issue is under question see section 2.

The representative use evaluated is on outdoor grapes as a foliar spray to control grape vine moth and grape berry moth. Full details of the GAP can be found in the list of end points in Appendix A-3.

CONCLUSIONS OF THE EVALUATION

1. Identity of the micro-organism/biological properties/physical and technical properties and methods of analysis.

Bacillus thuringiensis subsp. kurstaki strain ABTS-351 is deposited in the Safe Deposit storage facilities of the American Type Culture Collection, Rockville, MD under the identification number ATCC-SD-1275.

Bacillus thuringiensis subsp. kurstaki strain PB54 is deposited in the Spanish Collection of Cultures Type (CECT) under the registration number CECT 7209

It was concluded that the following 3 strains are very similar and in the main could be considered together. Strain SA-11, SA-12 and EG2348 are all deposited in the ARS Culture Collection (also known as Northern National Research Laboratory (NRRL), at the Microbial Properties Research Unit, National Centre for Agricultural Utilisation Research, Agricultural Research Service, U.S. Department of Agriculture Peoria, Illinois 61604. The registration numbers are NRRL B-30790, NRRL B-30791 and NRRL B-18208.

The strains are not human pathogens and are not related to human pathogens.

The strains may produce enterotoxins, β-exotoxin and cytolytic proteins and these may be present in the formulated product, a data gap was identified to address this issue. The content of contaminating micro-organisms was not fully addressed and a data gap was identified.

A method of analysis to unequivocally identify the organisms down to strain level was not available to the peer review. All the methods of analysis were not validated for the strains, contaminating and

pathogenic micro-organisms and toxins. It was considered that a method of analysis for residues in plants is needed and a data gap was identified for this.

For the WP formulation containing strain EG2348 there was no data on physical and chemical properties or product storage stability and a data gap was identified.

For the formulation 'Belthirul' containing PB54 the following data gaps were identified shelf life study, suspensibility, sprayability study to demonstrate that the formulation will not block spray nozzles.

2. Mammalian toxicity

General data on *Bacillus thuringiensis*

In the open literature, cases of human illness may have been underreported because of the close relationship to the human pathogen *Bacillus cereus*. Only the production of insecticidal crystal proteins by *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 food borne poisoning related to the *Bacillus cereus* type toxins (enterotoxins) susceptible to be produced by *Bacillus thuringiensis kurstaki*.

Observations in workers showed that exposure to sprays of *Bacillus thuringiensis subsp. kurstaki* may lead to allergic skin sensitisation and induction of antibodies. Upper airway and gastrointestinal symptoms were observed in populations exposed to aerial spraying of bio-insecticides based on *Bacillus thuringiensis subsp. kurstaki*, however the validity of the findings was debatable; in addition, the relationship with *Bacillus thuringiensis subsp. kurstaki* was not clearly demonstrated.

Bacillus thuringiensis kurstaki ABTS-351 / SA-11/SA-12/EG-2348 / PB-54

The strain PB-54 has been discussed in the expert meeting on mammalian toxicology in June 2009 (PRAPeR meeting M3); the strains ABTS-351 and SA-11/SA-12/EG-2348 have been discussed in May 2011 (Pesticides Peer Review meeting M4).

No information has been provided on the potential transfer of genetic material from Bacillus thuringiensis 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 on human and animal health has to be provided.

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).

Additionally, the test material used in the toxicology studies for the two formulations with the strain SA-11 has to be checked for its compliance to the formulations containing the strains SA-12 and EG-2348.

Toxicity studies

The data regarding infectivity and clearance were extrapolated between the different strains, considering the common low acute oral toxicity observed (at doses ranging from 10^8 to 10^{11} CFU/rat). Taking into account the low toxicity after repeated exposure with ABTS-351 (13-week dietary study at a dose $>10^9$ spores/animal/day; 2-year rat study at a dose $> 10^{10}$ spores/animal/day) or SA-11 (13-week at a dose of 10^8 CFU/rat), it was concluded that the lack of investigation of the clearance and infectivity in several studies was not a concern for the human risk assessment. This is also supported by a literature study where *Bacillus thuringiensis subsp. kurstaki* was administered orally to monkeys ($\sim10^9$ CFU/animal), showing no adverse effect and excretion of *Bacillus thuringiensis subsp. kurstaki* in faeces for approximately 2 weeks after administration.

In the intratracheal study with ABTS-351 (10^8 CFU/rat), the experts assumed that an immunological reaction was the cause of the high mortality and lung lesions in treated animals. In the inhalation study (10^8 CFU/rat), no mortality or immunological reaction was observed and the experts agreed that this was related to a lower dose reaching the deep lung after inhalation. The intratracheal (and intranasal) route of administration is considered less relevant for the human risk assessment than the inhalation exposure.

In the available 4-week inhalation study with guinea pigs, the relevant NOAEL was 1.2 x 10^7 CFU of ABTS-351/L based on an increased incidence and severity of lung lesions at 1.2 x 10^8 CFU/L.

An acute inhalation study with SA-11 (3.4 x 10^7 CFU/L) showed only transient clinical signs in rats, on the day of treatment; no inhalation study was provided for PB-54. Considering the available results and data, the experts agreed to extrapolate the toxicity by inhalation between the different strains of *Bacillus thuringiensis subsp. kurstaki*.

The intravenous administration of ABTS-351 (10^7 CFU/rat) did not give clear indication of pathogenicity relevant for the human risk assessment. No adverse effects were observed after intravenous administration of SA-11 (~10^8 CFU/kg bw) to rats. After intraperitoneal administration of ABTS-351 (10^8 CFU/mouse) or PB-54 (10^7 CFU/rat), no adverse effects were observed.

The warning phrase for the sensitisation potential of microbials: "Micro-organisms may have the potential to provoke sensitising reactions" was agreed for all *Bacillus thuringiensis subsp. kurstaki*.

No genotoxicity studies have been submitted for ABTS-351 and PB-54. No genotoxic effects were observed with SA-11, in an Ames test, chromosome aberration test and micronucleus test. It is noted that no validated method for genotoxicity testing of microorganisms is available. If toxins are confirmed to be present/produced (see data gap in section 1), the need for further assessment of their genotoxic potential will have to be considered.

Considering that the WP formulation containing the strain EG2348 is likely to be dusty, the acute inhalative toxicity of the product should be addressed. Additionally, the acute oral and inhalative toxicity of the WP formulation containing the strain PB-54 has also to be addressed.

Reference values

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

The potential of food borne poisoning, related to the *Bacillus cereus* type toxins (enterotoxins) susceptible to be produced by *Bacillus thuringiensis subsp. kurstaki*, has to be taken into account. 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

The exposure models are not appropriate for micro-organisms. Due to the sensitisation potential of micro-organisms, the use of adequate personal protective equipment should be further considered for dermal and inhalatory exposure. For the bystanders, the exposure cannot be prevented by the use of personal protective equipment. Nevertheless, the weight of evidence does not support the setting of a critical area of concern. Additionally, due to the data gap in section 1 for analysis of the potential toxins, the operator, worker and bystander risk assessment cannot be concluded.

3. Residues

The active components of commercial *B. thuringiensis ssp. kurstaki* preparations, spores and crystal proteins, are not toxic or pathogenic to humans, plants, and most animals except for larvae of target and non-target species belonging to the insect order Lepidoptera. This is to be confirmed by a data gap for batch analysis for possible toxins formed in the production process.

The only remaining issue for consumer exposure is that *B. thuringiensis ssp*. carry the genetic material that encodes for the *Bacillus cereus* enterotoxin it is not known if this can be expressed and under what conditions.

In a 2005 EFSA opinion on *Bacillus cereus* it was presented that food poisoning incidents in rare cases were caused by levels of 10^3 CFU/g of food.

For strain ABTS-351 use on cabbages the rapporteur calculated that the residue at harvest would be $6.62x10^4$ CFU/g however that calculation used a yield of 680 dt/ha if a more realistic yield is used then the figure is $2.25x10^6$ CFU/g.

For strain PB54 use on glass house tomatoes the calculated concentration is 2.3×10^4 CFU/g however this may be an underestimate as it is not clear what yield figure is used. If the yield figure is for annual glass house production per hectare the concentration per gram of tomato would be much higher as the glass house tomatoes are continuously cropped.

For strains SA-11, SA-12 and EG2348 use on grapes the calculated concentration is 5.6×10^6 CFU/g.

All of the calculated concentrations exceed 10^3 CFU/g where some poisoning incidents were seen for *Bacillus cereus*. Therefore to refine the risk assessment a data gap for residue trials has been identified. The residue trials will need to address the residues at harvest but also at the point of consumption as it is possible the levels of CFU could increase during transport, processing etc. The data provided must be strain specific.

4. Environmental fate and behaviour

Fate and behaviour in the environment of the micro-organism

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).

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).

Single application rate of 0.04-0.08 Kg a.s /ha is approximately equivalent to an application of 4×10^{11} CFU / kg of *Bacillus thuringiensis* subsp *kurstaki* PB54. Up to 12 applications are foreseen for the representative use in tomatoes. A general conversion factor of the IU (international units), reported in some of the studies provided, to mass amount or CFU counts was deemed not possible by the experts in the M3 meeting. This conversion may need to be considered on a case by case basis for each of the studies (see point of clarification8.2 in PB-54 evaluation table). The application rate calculated based on the amount of crystalline proteins may need to be considered in order to finalise the environmental assessment of the active metabolites (see open point 8.9 in PB-54 evaluation table). Whereas this issue was identified during the discussion of the strain PB-54, it applies by analogy to all strains discussed in this conclusion.

No studies on **persistence and multiplication in soil** of *Bacillus thuringiensis* subsp. *kurstaki* strains PB54, ABTS-351, SA-11, SA-12 and EG-2348 are available. Assessment presented in the DARs is based on the scientific publications submitted 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. However, no individual assessment of the publications was presented in the DARs, it being difficult to

track back the statements and conclusions to the original scientific data (see open point 8.3 in PB-54 evaluation table). During the peer review a number of issues have been considered to require further clarification or data. A tabulated summary of some data available in the literature related to the persistence of the spores of *Bacillus thuringiensis* subsp. *kurstaki* under field conditions may be found in the addenda to the DARs provided by the RMS.

According the information provided by the applicants, vegetative cells are not expected to occur in the marketed products of *Bacillus thuringiensis* subsp. *kurstaki*. Vegetative cells are expected to survive and multiply in soil and dead insect cadavers under favourable conditions. In the DARs the persistence and multiplication of vegetative cells in soil is stated to occur only under a narrow range of favourable conditions and limited in time. Among the factors that may have an effect on the survival of *Bacillus thuringiensis* in soil: temperature, pH, moisture, soil type, presence of other microorganisms and light are listed. 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. *kurstaki*. 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. *kurstaki* 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 only been calculated for *Bacillus thuringiensis* subsp. *kurstaki strain* PB54 and may be found in the corresponding addendum.

No studies on **persistence and multiplication in water** of *Bacillus thuringiensis* subsp. *kurstaki strains* PB54, ABTS-351, SA-11, SA-12 and EG-2348 are available. Assessment presented in the DARs is based on the scientific publications submitted by the applicants in the dossiers on other strains and or species of the *Bacillus thuringiensis*. However, no individual assessment of the publications was presented in the DARs, it being 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}. These can be found in the corresponding DARs for strains ABTS-351, SA-11, SA-12 and EG-2348 and in the addendum for strain PB54.

Fate and behaviour in the environment of any relevant metabolite formed by the micro-organism 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 specie to specie and among different strains. The variations usually result in proteins selective to different kind of insects. *Bacillus thuringiensis* subsp. *kurstaki* crystalline proteins are recognised to be selective to larvae of insects of the *Lepidoptera* order. 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. *kurstaki strains* PB54, ABTS-351, SA-11, SA-12 and EG-2348 crystalline proteins are available in the dossiers. Assessment presented in the DAR is based on the 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 species 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 DARs 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, it being difficult to track back the statements and conclusions to the original scientific data. Worst case estimations of soil had water halve lives and soil adsorption would be needed to finalise the environment exposure assessment of these proteins. Whereas it is claimed that no risk of **contamination of groundwater** exists, scientific data and calculations supporting such statements are not transparently reported in the DAR. 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.

Experimental information on the persistence of the crystal protein in **surface water** is not transparently reported in the DARs. 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 can be found in the corresponding DARs for strains ABTS-351, SA-11, SA-12 and EG-2348 and in the addendum for strain PB54.

5. Ecotoxicology

Strain PB-54.

The studies presented on non target organisms are based on a literature review carried out by the notifier. No specific data were provided on PB-54. However, it was agreed that extrapolation between different *Bacillus thuringiensis kurstaki* strains can be considered acceptable for non-target organisms, except for daphnids and non-target arthropods. For *Daphnia* the extrapolation was considered uncertain for technical products while for non-target arthropods more justification would be needed to demonstrate whether or not extrapolation is appropriate (see below the data gaps identified).

On the basis of the available data, no pathogenicity and toxicity was observed for birds and mammals. A low risk was assessed for the indicator species medium herbivorous and insectivorous birds. The potential exposure following the consumption of contaminated larvae, to vegetative cells and metabolites/toxins produced during the vegetative growth was considered as low. Indeed, the experts agreed that level of toxins on insects, if present, would be low. Also the risk to mammals can be considered as low.

No pathogenicity and toxicity was observed for aquatic organisms and a low risk was indicated by TER values provided with the addendum (September 2011). A data gap was identified for a strain specific study on *Daphnia magna*. Alternatively a robust argumentation would be needed to support the extrapolation from other Btk strains data.

The off-crop risk for non-target Lepidoptera was addressed by RMS following the HQ approach. This risk assessment was included in an addendum provided after the experts' consultation at PRAPeR M4 (May 2011). However, the HQ approach cannot be used to quantitatively address the risk assessment for species other than standards ones (i.e *T.piry* and *A. rhopalosiphi*). Additionally, in view of the restrictions concerning the acceptance of new (i.e. newly submitted) studies after the submission of the DAR to EFSA, as laid down in Commission Regulation (EC) No 1095/2007, new studies could not be considered in the peer review, unless they indicate more concern. Therefore a data gap is identified to provide the papers mentioned in the addendum on the acute toxicity of *Bacillus thuringiensis kurstaki* to Lepidoptera. It is, however, noted that the drift exposure rate would be lower than the lethal rate, anticipating a low risk for non-target Lepidoptera. A data gap was also agreed at PRAPeR M3 (June 2009) to provide a justification whether or not extrapolation on arthropod effect data from other strains is appropriate.

A low risk is expected to bees, earthworms, soil macro and micro organisms, non target plants and methods for sewage treatment plants.

Strains SA-11, SA-12, EG2348

Ecotoxicological studies were provided for SA-11 and EG2348 but no data were submitted for SA-12. However, as agreed for PB-54, the extrapolation between different *Bacillus thuringiensis kurstaki* strains can be considered acceptable for non-target organisms, except for daphnids and non-target arthropods (see below the data gaps identified).

On the basis of available data, no pathogenicity and toxicity was observed for birds and mammals. No TER calculations were provided, but the risk could be considered as low for the indicator species in vine/orchard scenario. The potential exposure following the consumption of contaminated larvae, to vegetative cells and metabolites/toxins produced during the vegetative growth was considered as low. Indeed, the experts agreed that level of toxins on insects, if present, would be low. Indeed, the experts agreed that level of toxins on insects, if present, would be low. Also the risk to mammals can be considered as low.

No pathogenicity and toxicity was observed for aquatic organisms and a low risk was indicated by comparing the toxicity endpoint with the PECsw. A data gap was identified for a specific study on *Daphnia magna* with the strain SA-12, due to the uncertainties on the extrapolation between different *Bacillus thuringiensis kurstaki* strains for technical products. Alternatively a robust argumentation would be needed to support the extrapolation from other Btk strains data.

The off-crop risk for non-target Lepidoptera was addressed by RMS following the HQ approach. This risk assessment was included in an addendum provided after the experts' consultation at PRAPeR M4 (May 2011). However, the HQ approach cannot be used to quantitatively address the risk assessment for species other than standards ones (i.e *T.piry* and *A. rhopalosiphi*). Additionally, in view of the restrictions concerning the acceptance of new (i.e. newly submitted) studies after the submission of the DAR to EFSA, as laid down in Commission Regulation (EC) No 1095/2007, new studies could not be considered in the peer review, unless they indicate more concern. Therefore a data gap is identified to provide the papers mentioned in the addendum on the acute toxicity of *Bacillus thuringiensis kurstaki* to Lepidoptera. It is, however, acknowledged that the drift exposure rate would be lower than the lethal rate, anticipating a low risk for non-target Lepidoptera. A data gap was also agreed at PRAPeR M3 (June 2009) to provide a justification whether or not extrapolation on arthropod effect data from other strains is appropriate.

A low risk is expected to bees, earthworms, soil macro and micro organisms, non target plants and methods for sewage treatment plants.

Strain ABTS-351

On the basis of available data, no pathogenicity and toxicity was observed for birds and mammals. A low risk was assessed for the indicator species medium herbivorous and insectivorous birds. The potential exposure following the consumption of contaminated larvae, to vegetative cells and metabolites/toxins produced during the vegetative growth was considered as low. Indeed, the experts agreed that level of toxins on insects, if present, would be low. Indeed, the experts agreed that level of toxins on insects, if present, would be low. Also the risk to mammals can be considered as low.

No pathogenicity and toxicity was observed for aquatic organisms and a low risk was indicated by the TERs values. A new study was submitted on *Daphnia magna* and evaluated by the RMS in the addendum. This study was considered, even if provided in a later stage of the peer review because the endpoint was slightly lower than the endpoint available in the DAR.

The off-crop risk for non-target Lepidoptera was addressed by RMS following the HQ approach. This risk assessment was included in an addendum provided after the experts' consultation at PRAPeR M4 (May 2011). However, the HQ approach cannot be used to quantitatively address the risk assessment for species other than standards ones (i.e *T.piry* and *A. rhopalosiphi*). Additionally, in view of the restrictions concerning the acceptance of new (i.e. newly submitted) studies after the submission of the DAR to EFSA, as laid down in Commission Regulation (EC) No 1095/2007, new studies could not be considered in the peer review, unless they indicate more concern. Therefore a data gap is identified to provide the papers mentioned in the addendum on the acute toxicity of *Bacillus thuringiensis kurstaki* to Lepidoptera. It is, however, acknowledged that the drift exposure rate would be lower than the lethal rate, anticipating a low risk for non-target Lepidoptera.

A low risk is expected to bees, non-target arthropods, earthworms, soil macro and micro organisms, non target plants and methods for sewage treatment plants.

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, viability and dynamics	Ecotoxicology
Bacillus thuringiensis subsp. *kurstaki strains* PB54 spores	May persist months to years in soil	Low risk identified
Bacillus thuringiensis subsp. *kurstaki strains* PB54 crystalline proteins (δ endotoxins).	No conclusive data available.	Low risk identified

Compound (name and/or code)	Persistence, viability and dynamics	Ecotoxicology
Bacillus thuringiensis subsp. *kurstaki strain* ABTS-351 spores	May persist months to years in soil	Low risk identified
Bacillus thuringiensis subsp. *kurstaki strain* ABTS-351 crystalline proteins (δ endotoxins).	No conclusive data available.	Low risk identified

Compound (name and/or code)	Persistence, viability and dynamics	Ecotoxicology
Bacillus thuringiensis subsp. *kurstaki strain* SA-11 spores	May persist months to years in soil	Low risk identified
Bacillus thuringiensis subsp. *kurstaki strain* SA-11 crystalline proteins (δ endotoxins).	No conclusive data available.	Low risk identified

Compound (name and/or code)	**Persistence, viability and dynamics**	**Ecotoxicology**
Bacillus thuringiensis subsp. *Kurstaki strains* SA-12 spores	May persist months to years in soil	Low risk identified
Bacillus thuringiensis subsp. *Kurstaki strains* SA-12 crystalline proteins (δ endotoxins).	No conclusive data available.	Low risk identified

Compound (name and/or code)	**Persistence, viability and dynamics**	**Ecotoxicology**
Bacillus thuringiensis subsp. *Kurstaki strains* EG-2348 spores	May persist months to years in soil	Low risk identified
Bacillus thuringiensis subsp. *kurstaki strains* EG-2348 crystalline proteins (δ endotoxins).	No conclusive data available.	Low risk identified

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. *Kurstaki strains* PB54 crystalline proteins (δ endotoxins).	No data available	No data available. Data gap	yes	Yes	Low risk identified

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. *kurstaki strain* ABTS-351 crystalline proteins (δ endotoxins).	No data available	No data available. Data gap	yes	Yes	Low risk identified

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. *Kurstaki strain* SA-11 crystalline proteins (δ endotoxins).	No data available	No data available. Data gap	yes	Yes	Low risk identified

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. *kurstaki strains* SA-12 crystalline proteins (δ endotoxins).	No data available	No data available. Data gap	yes	Yes	Low risk identified

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. *kurstaki strains* EG-2348 crystalline proteins (δ endotoxins).	No data available	No data available. Data gap	yes	Yes	Low risk identified

6.3. Surface water and sediment

Compound (name and/or code)	Ecotoxicology
Bacillus thuringiensis subsp. *kurstaki strains* PB54 spores	Low risk identified
Bacillus thuringiensis subsp. *kurstaki strains* PB54 crystalline proteins (δ endotoxins).	Low risk identified

Compound (name and/or code)	Ecotoxicology
Bacillus thuringiensis subsp. *kurstaki strains* ABTS-351 spores	Low risk identified
Bacillus thuringiensis subsp. *kurstaki strains* ABTS-351 crystalline proteins (δ endotoxins).	Low risk identified

Compound (name and/or code)	**Ecotoxicology**
Bacillus thuringiensis subsp. *kurstaki strains* SA-11 spores	Low risk identified
Bacillus thuringiensis subsp. *kurstaki strains* SA-11 crystalline proteins (δ endotoxins).	Low risk identified

Compound (name and/or code)	**Ecotoxicology**
Bacillus thuringiensis subsp. *kurstaki strains* SA-12 spores	Low risk identified
Bacillus thuringiensis subsp. *kurstaki strains* SA-12 crystalline proteins (δ endotoxins).	Low risk identified

Compound (name and/or code)	**Ecotoxicology**
Bacillus thuringiensis subsp. *kurstaki strains* EG-2348 spores	Low risk identified
Bacillus thuringiensis subsp. *kurstaki strains* EG-2348 crystalline proteins (δ endotoxins).	Low risk identified

6.4. Air

Compound (name and/or code)	**Toxicology**
Bacillus thuringiensis subsp. *kurstaki strains* PB54 spores	Low acute toxicity via inhalation
Bacillus thuringiensis subsp. *kurstaki strains* PB54 crystalline proteins (δ endotoxins).	No data available

Compound (name and/or code)	**Toxicology**
Bacillus thuringiensis subsp. *kurstaki strains* ABTS-351 spores	Low acute toxicity via inhalation
Bacillus thuringiensis subsp. *kurstaki strains* ABTS-351 crystalline proteins (δ endotoxins).	No data available

Compound (name and/or code)	**Toxicology**
Bacillus thuringiensis subsp. *kurstaki strains* SA-11 spores	Low acute toxicity via inhalation
Bacillus thuringiensis subsp. *kurstaki strains* SA-11 crystalline proteins (δ endotoxins).	No data available

Compound (name and/or code)	**Toxicology**
Bacillus thuringiensis subsp. *kurstaki strains* SA-12 spores	Low acute toxicity via inhalation
Bacillus thuringiensis subsp. *kurstaki strains* SA-12 crystalline proteins (δ endotoxins).	No data available

Compound (name and/or code)	**Toxicology**
Bacillus thuringiensis subsp. *kurstaki strains* EG-2348 spores	Low acute toxicity via inhalation
Bacillus thuringiensis subsp. *kurstaki strains* EG-2348 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).

- Batch data should be provided to show compliance with the OECD issue Paper dated February 2011 'Discussion on Microbial Contaminant limits for Microbial Pest Control Products'. The methods used must be validated (relevant for all representative uses evaluated; submission date proposed by the notifier: unknown; see section 1)

- Method of analysis for the unequivocal identification of each strain (relevant for all representative uses evaluated; submission date proposed by the notifier: unknown; see section 1)

- The references that have not been submitted as detailed in the August addendum are identified as a data gap. (relevant for strains ABTS-351, SA-11, SA-12 and EG2348; submission date proposed by the notifier: unknown; see section 1)

- The references that have not been submitted as detailed in the September addendum are identified as a data gap. (relevant for strain PB54; submission date proposed by the notifier: unknown; see section 1)

- 3-5 batch analyses to exclude the presence of enterotoxins (B.cereus type: haemolytic, non haemolytic or cytotoxic); beta-exotoxin (ATP analogue); cytolytic proteins (parasporins acting in combination with ICP) above a detection limit of at least 1 mg/kg. Experimental evidence that pertinent genes are absent from the strain might also address this. (relevant for all representative uses evaluated; submission date proposed by the notifier: unknown; see section 1)

- Validated methods of analysis (relevant for all representative uses evaluated; submission date proposed by the notifier: unknown; see section 1)

- Validated method for *B. anthracis* (relevant for all representative uses evaluated; submission date proposed by the notifier: unknown; see section 1)

- For the WP formulation containing strain EG2348 a phys/chem. properties and storage stability data package (relevant for strain EG2348; submission date proposed by the notifier: unknown; see section 1)

- The protein with a molecular mass of app. 97 kDa must be identified. The individual Cry1 and Cry2 toxins in the product must be identified and quantified. The individual δ-endotoxin genes present in *B. thuringiensis* PB54 should be identified (relevant for strain PB54; submission date proposed by the notifier: unknown; see section 1)

- For the formulation 'Belthirul' shelf life study, suspensibility and a sprayability study under field conditions with pertinent equipment to confirm that there would not be an issue with the blockage of nozzles (relevant for strain PB54; submission date proposed by the notifier: unknown; see section 1)

- The test material used in the toxicology studies for the two formulations with the strain SA-11 has to be checked for its compliance to the formulations containing the strains SA-12 and EG-2348 (relevant for strain SA-11; no submission data proposed by the notifier; see section 2)

- Considering that the WP formulation containing the strain EG2348 is likely to be dusty, the acute inhalative toxicity of the product should be addressed (relevant for strain EG2348, no submission data proposed by the notifier; see section 2)

- The acute oral and inhalative toxicity of the WP formulation containing the strain PB-54 has to be addressed (relevant for strain PB-54, no submission data proposed by the notifier; see section 2)

- Strain specific residue data to show the level of CFU on the crop at harvest and when consumed. (relevant for all representative uses evaluated; submission date proposed by the notifier: unknown; see section 3)

- 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)

- For all the strains under examination (PB-54, ABTS-351, SA-11, SA-12 and EG-2348), a data gap has been identified to demonstrate that, under the conditions of use, *Bacillus thuringiensis* subsp. *kurstaki* 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. (relevant for all representative uses evaluated; submission date proposed by the notifier: no submission date provided; see section 4)

- Strain specific studies on *Daphnia magna.* for the strain PB-54, SA-12 or alternatively a robust argumentation would be needed to support the extrapolation from other Btk strains data. (relevant for the field representative uses on grapes and tomatoes; submission date proposed by the notifier: unknown; see section 5);

- Information/justification should be provided to demonstrate whether or not extrapolation on arthropod effects data from other strains is appropriate for the strains PB-54, SA-11, SA-12 and EG2348 (relevant for the field representative uses on grapes and tomatoes; submission date proposed by the notifier: unknown; see section 5).

- A data gap was also identified to provide the papers mentioned in the addendum on the acute toxicity of *Bacillus thuringiensis kurstaki* to Lepidoptera, used to address the off-crop risk to non-target Lepidoptera population for all the strains under examination (PB-54, ABTS-351, SA-11, SA-12 and EG-2348), (relevant for all the field representative uses evaluated ; submission date proposed by the notifier: unknown; see section 5);

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

- None.

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. 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.

2. No information has been provided on the potential transfer of genetic material from *Bacillus thuringiensis* to other organisms. Assessment of potential transfer of genetic material and its effects on the environment cannot be finalized.

3. Assessment of potential contamination of groundwater by *Bacillus thuringiensis* subsp. *kurstaki* crystalline proteins (δ endotoxins) with respect to the regulatory limit of 0.1 µg /L is not available for any of the strains PB-54, ABTS-351, SA-11, SA-12 and EG-2348 .

4. The operator, worker and bystander risk assessment cannot be finalised as it is not clear if *Bacillus thuringiensis* subsp. *kurstaki* can produce enterotoxin.

5. The consumer risk assessment cannot be finalised as it is not clear if *Bacillus thuringiensis* subsp. *kurstaki* can produce enterotoxin.

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 assessments 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.) All columns are grey due to the lack of information on the toxins and contaminating micro-organisms.

Representative use *Bacillus thuringiensis* subsp. *kurstaki* strain PB-54		**Grapes (SA-11, SA-12, EG 2348)**	**Cabbage (ABTS-351)**	**Tomatoes (PB54)**
Operator risk	Risk identified			
	Assessment not finalised	X[4]	X[4]	X[4]
Worker risk	Risk identified			
	Assessment not finalised	X[4]	X[4]	X[4]
Bystander risk	Risk identified			
	Assessment not finalised	X[4]	X[4]	X[4]
Consumer risk	Risk identified			
	Assessment not finalised	X[5]	X[5]	X[5]
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			
Groundwater exposure active substance	Legal parametric value breached			
	Assessment not finalised			
Groundwater exposure metabolites	Legal parametric value breached			
	Parametric value of 10µg/L[(a)] breached			
	Assessment not finalised	X[3]	X[3]	X[3]

The superscript numbers in this table relate to the numbered points indicated as concerns

(a): Value for non relevant metabolites prescribed in SANCO/221/2000-rev 10-final, European Commission, 2003

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European Commission, 2008b. Review Report for the active substance *Bacillus thuringiensis subsp. kurstaki* (strain PB 54) 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 subsp. kurstaki* (strain PB 54) in Annex I of Directive 91/414/EECSANCO/1542/08 – rev. 3, 30 June 2008

European Commission, 2008c. Review Report for the active substance *Bacillus thuringiensis subsp. kurstaki* (strain ABTS 351) 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 subsp. kurstaki* (strains SA 11, SA 12, EG 2348) in Annex I of Directive 91/414/EECSANCO/1541/08 – rev. 3, 7 May 2008

European Commission, 2003. Guidance Document on Assessment of the Relevance of Metabolites in Groundwater of Substances Regulated under Council Directive 91/414/EEC. SANCO/221/2000-rev. 10 - final, 25 February 2003.

APPENDICES

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

BACILLUS THURINGIENSIS KURSTAKI ABTS 351

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

Active micro-organism	*Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351
Function (*e.g.* fungicide)	Insecticide

Identity of the micro-organism (Annex IIM 1)

Name of the organism:	*Bacillus thuringiensis*
Taxonomy:	Bacillaceae: Bacillus
Species, subspecies, strain:	*Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351
Identification:	The strain is derived from *Bacillus thuringiensis* subsp. *kurstaki* strain HD-1, however HD-1 and APTS-351 are synonymus throughout the DAR. Confidential information, see Volume 4
Culture collection:	ATCC SD-1275
Minimum and maximum concentration of the micro-organism used for manufacturing of the formulated product (cfu/g; cfu/L, etc.):	1.17×10^{10} CFU/g nominal concentration
Identity and content of relevant impurities in the technical grade micro-organism:	Open
Is the MCPA genetically modified; if so provide type of modification	No modification

Biological properties of the micro-organism (Annex IIM)

Origin and natural occurrence, background level:	ABTS-351 is a naturally occurring strain derived from a diseased Pink Bollworm. *B. thuringiensis* subsp. *kurstaki* is ubiquitous in many environments such as insects, soil and plant surfaces
Target organism(s):	Larvae of Cabbage Moth (*Mamestra brassicae*)
Mode of action:	The crystal proteins of *B. thuringiensis* must be ingested to be effective against the target insect. Upon ingestion of *B. thuringiensis* by the larvae, the crystalline inclusions dissolve in the larval midgut, releasing insecticidal crystal proteins. The activated Cry toxins interact with the midgut epithelium cells of susceptible insects. After binding to the midgut receptors, they insert into the apical membrane to create ion channels, or pores, disturbing the osmotic balance and permeability. This can result in colloid-osmotic lysis of the cells. Spore germination and proliferation of vegetative cells into the haemocoel may result in septicaemia, contributing to mortality of the insect larvae.
Host specificity:	The strain ABTS-351 is highly specific against insect species of the order *Lepidoptera*
Life cycle:	Under beneficial conditions regarding moisture, temperature and nutrients, the basic metabolizing cell type is the vegetative cell that is actively growing and dividing. When a population of vegetative cells passes out of the exponential phase of growth, usually as a result of nutrient depletion, the differentiation of endospores begins. During sporulation, *B. thuringiensis* produces inclusion bodies that are composed of Cry proteins. Endospores will germinate and form vegetative cells, when favourable conditions return for the growth of these cells. Before germination an endospore can pass through an extended period of dormancy, and is not able to replicate itself or form other spores.
Infectivity, dispersal and colonisation ability:	*B. thuringiensis* subsp. *kurstaki* strain ABTS-351 is specific to several species of insects of the order *Lepidoptera* and no cases of infectivity in other animal organisms or in plants is reported. *B. thuringiensis* subsp. *kurstaki* can be dispersed by the movement of insects and other animals carrying the bacteria in or on their bodies. The bacterium has poor colonization ability and is not a good competitor in the soil. Its survival is dependent on the presence and activity of other soil microorganisms and protection from degradation effects of sunlight. Applied as a spray on above ground leaves and fruits, endospores are rapidly inactivated and δ-endotoxins are rapidly degradable when exposed to UV-radiation.

Relationship to known pathogens:	*B. thuringiensis* is a member of the *Bacillus cereus* group which comprises closely related Gram positive bacteria that exhibit highly divergent pathogenic properties, including two human pathogenic species, *B. cereus* and *Bacillus anthracis*. *B. cereus* can cause food poisoning by the production of toxins, whereas *B. anthracis* is the causative agent of anthrax. A range of studies on small mammals has shown a very low risk from direct exposure to Bt spores and δ-endotoxins.
Genetic stability:	During the production process the Btk ABTS-351 strain is proved to be stable by regular quality control checks. The spontaneous loss of plasmids, carrying the information for the insecticidal active crystal proteins, would be detected in bioassays. The transfer of genetic material in the fermentation broth is very unlikely due to the absence of microbial impurities and the continuous stirring movements of the fermentation broth. The quality control of the production batches confirms the stability of the *B. thuringiensis kurstaki* strain ABTS-351.
Production of relevant metabolites/toxins:	Open
Resistance/sensitivity to antibiotics/anti-microbial agents used in human or veterinary medicine:	The strain ABTS-351 is intrinsically resistant to Penicillin, Ampicillin and Cephalothin. It is susceptible to Gentamicin, Kanamycin, Erythromycin, Clindamycin, Vancomycin, Chloramphenicol and Trimethoprim/Sulfamethoxazole.

Classification and proposed labelling

with regard to the micro-organism:	Microorganisms may have the potential to provoke sensitising reactions

Summary of representative uses evaluated

Crop and/ or situation (a)	Member State or Country	Product name	F G or I (b)	Pests or Group of pests controlled (c)	Preparation		Application				Application rate per treatment			PHI (days) (m)	Remarks
					Type (d-f)	Conc. of MPCA (i)	Method kind (f-h)	Growth stage & season (j)	Numb er min/ max (k)	Interval between applications (min)	Kg MPCA/ hL; cfu/hL min – max (l)	Water L/ha min – max	Kg MPCA/ha; cfu/ha min – max (l)		
Cabbage	Europe	DiPel WG	F	*Mamestra brassicae, and otzher defoliating lepidopteran pests* (Cabbage Moth)	WG	54% Approx $1{,}17 \times 10^{13}$ CFU/kg	Spray	Primarily against first generation of pests/start the the first eggs hatching. Possible against second generation of pests	3-8	7 days	0.054 kg – 0.100 kg/hL	400-1000 L/ha	0.405 kg – 0.54 kg/ha $4.7\text{-}6.3 \times 10^{12}$ CFU/ha	0	0.75-1.00 kg product/ha. Potency 32000 IU/mg product

* For uses where the column "Remarks" is marked in grey further consideration is necessary. Uses should be crossed out when the notifier no longer supports this use(s).
(a) For crops, the EU and Codex classifications (both) should be taken into account; where relevant, the use situation should be described (e.g. fumigation of a structure)
(b) Outdoor or field use (F), greenhouse 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/kg or cfu/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) The values should be given in g or kg whatever gives the more manageable number (e.g. 200 kg/ha instead of 200 000 g/ha or 12.5 g/ha instead of 0.0125 kg/ha) as well as in number of cfu
(m) PHI - minimum pre-harvest interval

Chapter 2 Analytical methods

Analytical methods for the micro-organism (Annex IIM 4.2, 4.3; IIIM 5.4)

Manufactured micro-organism (principle of method):	Open
Impurities and contaminating micro-organisms in manufactured material (principle of method):	Open
Microbial plant protection product (principle of method):	Open

Analytical methods for residues (viable and non-viable) (Annex IIM 4.5)

of the active micro-organism (principle of method):	Open
of relevant metabolites (principle of method):	Open

Chapter 3 Impact on Human and Animal Health (Annex IIM 5; IIIM 7)

Chapter 3 Impact on Human and Animal Health (Annex IIM 5; IIIM 7)

Medical data, surveillance and observations:	Only in a few cases *Bt* has been isolated from human bacterial infection. One case of corneal ulcer after accidental splashing of a worker with *Btk* product. No evidence of adverse health effect among manufacturing plant personnel exposed to strain ABTS-351. No gastro-intestinal symptoms correlated with the presence of *Bt* in the faeces were observed in greenhouse workers spraying *Btk* ABTS-351. No major effects were observed in populations exposed to aerial spraying of *Btk* (upper airways and gastrointestinal symptoms) but no clear relationship could be established with *Btk*.
Sensitisation (experience in humans and study results; type of study):	Positive results in a dermal sensitization study (Magnusson Kligman method) of limited relevance for microbials. 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". Potential risk of skin sensitization and induction of IgE and IgG antibodies in persons exposed to *B. thuringiensis kurstaki*.

Toxicity	
after acute oral exposure:	NOAEL = 10^9 CFU/rat
after acute inhalation exposure:	Intratracheal study: high mortality at a dose of app. 10^8 CFU/rat. Similar results were obtained after intranasal administration of other *Bt* species. This was attributed to a mechanical/local effect not relevant for the human risk assessment. Inhalation study (4h exposure): rat LC_{50}>2.4 x 10^8 CFU/L
after acute intraperitoneal/subcutaneous exposure:	Intraperitoneal/subcutaneous: NOAEL= 10^8 CFU/mouse Intravenous: LOAEL= 10^7 CFU/rat

Infectivity	
after acute oral exposure:	Found in one spleen (1/6) and one kidney (1/6). High counts in faeces of all animals at Day 22. Slow clearance from the gastrointestinal tract is expected.
after acute inhalation exposure:	Intratracheal study: the bacteria was still present in lungs, spleen and liver at day 52. Inhalation study: clearance not investigated.
after acute intraperitoneal/subcutaneous exposure:	Intraperitoneal/subcutaneous: clearance not investigated. Intravenous study: the bacteria was present at significant high numbers in the spleen at day 155 (5/5).

Pathogenicity	
after acute oral exposure:	No evidence of adverse effects.
after acute inhalation exposure:	Intratracheal study: adverse effects in lungs (enlargement (29/66), red mottling (28/66), brown spots (13/66), grey mottling (5/66) and fluid filled trachea (1/66)). Inhalation study: no significant adverse effect
after acute intraperitoneal/subcutaneous exposure:	Intraperitoneal/subcutaneous: no adverse effects. Intravenous study : adverse effects on haematological parameters and multifocal granulomatous inflammation in lungs, liver, spleen and mediastinal lymph nodes.

Genotoxicity:	No validated methods available for microorganisms. Potential toxins might need to be further investigated for their genotoxic properties.

Cell culture study:	Non-specific cytotoxicity of *Btk* ABTS-351 for 7 human cell types. Results not relevant for *Btk* (which are not intracellular replicating bacteria).
Short term toxicity/pathogenicity:	13-wk rat, oral: no adverse effect at a dose $>10^9$ spores/animal/d; no data on infectivity/clearance 2-yr rat, oral: decreased body weight gain at a dose $>10^{10}$ spores/animal/d; no data on infectivity/clearance 4-wk, guinea pigs, inhalation: NOAEL of 1.2×10^7 CFU/L based on lung lesions at 1.2×10^8 CFU/L; no data on infectivity/clearance.
Specific toxicity, pathogenicity and infectiveness studies:	Potential toxins might need to be further investigated for their toxicological properties. Acute dermal study: rabbit $LD_{50} > 13.3 \times 10^{10}$ CFU/kg bw Skin irritation study: slightly irritating (erythema) Eye irritation study: transient conjunctival irritation
AOEL:	Not applicable. *Bacillus thuringiensis* strains cannot be excluded to produce enterotoxins (as *Bacillus cereus*) known to cause food poisoning in humans. In most instances, foodborne diseases caused by *Bacillus cereus* were associated with the intake of 10^5 to 10^8 CFU/g food. Lower numbers were reported in some outbreaks (10^3 to 10^4 CFU/g food).
ADI:	Not applicable. *Bacillus thuringiensis* strains cannot be excluded to produce enterotoxins (as *Bacillus cereus*) known to cause food poisoning in humans. In most instances, foodborne diseases caused by *Bacillus cereus* were associated with the intake of 10^5 to 10^8 CFU/g food. Lower numbers were reported in some outbreaks (10^3 to 10^4 CFU/g food).

Exposure scenarios (including method of calculation)

Application method:	Spraying of cabbage, in fields
Operator:	The exposure models for chemicals are not appropriate for micro-organisms. Based on a qualitative assessment (potential for sensitisation), the need of personal protective equipment (including respiratory) is highlighted for the operators.
Workers:	Based on the potential of microorganisms for sensitisation, the need of personal protective equipment is highlighted for re-entry workers.

Bystanders:	Based on the potential of microorganisms for sensitisation, the risk cannot be concluded for the bystanders during field use (no personal protective equipment can be foreseen).

Chapter 4 Residues

Residues on Treated Products, Food and Feed (Annex IIM 6; IIIM 8)

Non-viable residues:	Open
Viable residues:	Open

Chapter 5 Fate and behaviour in the environment (Annex IIM 7; IIIM 9)

Persistence and multiplication

In soil:	No data on the persistence and multiplication of *Bacillus thuringiensis* subsp. *kurstaki* strains ABTS-351-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. $\underline{PEC_{soil}}$ Application rate: 1 kg product Dipel WG/ha x 8= 8 kg Dipel WG / ha (equivalent to 4.32 MPCA/ha). Incorporation in soil; 5 cm Soil density of 1.5 g/cm3 Soil interception 0 %. The calculated initial PEC_{soil} is: ~~6.4~~ 10.7mg DiPel WG/kg dry weight soil corresponding to ~~3.46~~ 5.76 mg MPCA/kg dry weight soil and ~~7.5~~ 1.2 x 10^5 CFU/g soil.
In water:	No data on the persistence and multiplication of *Bacillus thuringiensis* subsp. *kurstaki* strains SA-11, SA-12 and EG 2348 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 calculation: the predicted initial concentration of DiPel WG in surface water following one application of 1kg of DiPel WG is 9.23 µg DiPel WG/L (4.98 µg MPCA/L) corresponding to 1.1 x 10^5 CFU/L. When a maximum of eight applications are considered (1kg DiPel WG x 8 = 8 kg DiPel WG) and no degradation over the intervening 49 days between the eight applications is assumed, the predicted initial concentration of DiPel WG in surface water is 73.84 µg

	DiPel WG/L (39.87 µg MPCA/L) corresponding to 8.5 x 10^5 CFU/L.
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.

Chapter 6 Effects on Non-target Organisms (Annex IIM 8; IIIM 10)

Effects on terrestrial vertebrates

Risk assessment for mammals:	The risk to mammals is assessed as low.
Effects on birds:	LD_{50} > 2857 mg bacteria/kg b.w. or 5.7 x 10^{10} spores/kg b.w./day
Risk assessment:	The risk to birds is assessed as negligible. TER values were above 40 and 70 for acute and short-term respectively and were thus well above the trigger value of 10.
Effects on other terrestrial vertebrates:	
Risk assessment:	The risk to terrestrial vertebrates was considered as low.

Effects on aquatic organisms

Effects on fish:	32-day LC_{50} > 143.5 mg MPCA/L or 2.87 x 10^9 CFU/L for Rainbow trout and Bluegill Sunfish
Risk assessment:	The risk to fish is assessed as low, TER > 3600 (trigger value 100)
Effects on freshwater invertebrates:	*Daphnia magna*: 21 day 5 < EC_{50} < 50 mg MPCP/L (1 x 10^8 < EC_{50} < 1 x 10^9 CFU/L NOEC < 5 mg MPCP/L *Daphnia magna*: LC_{50} < 100 mg MPCP/L *Daphnia magna*: 21 day EC_{50} =13 mg MPCP/L (3.8 x 10^5 CFU/L) based on mortality 21 day EC_{50} =7.8 mg MPCP/L (2.3x 10^5 CFU/L) based on reproduction. Harpacticoid copepod: LC_{50}> 500 mg MPCP/kg see water overlying sediment (1 x 10^{10} CFU/kg see water overlying sediment) NOEC 500 mg MPCP/kg see water overlying sediment Grass shrimp: LC_{50}> 2,87 x 10^9 CFU/g food; 1.7 x 10^7 CFU/L
Risk assessment:	The risk to freshwater invertebrates is assessed as negligible TER for *Daphnia magna* 125 - 1254 (Trigger value: 100)
Effects on algae:	72-hour EC_{50} = 50.8 mg MPCP/L, only stated as mg

Risk assessment:	The risk to algae is assessed as low TER 688 (Trigger value: 10)
Effects on aquatic plants:	No effects on aquatic plants from applications of strain ABTS-351 is expected or envisaged
Risk assessment:	The risk to aquatic plants is assessed as low

Effects on arthropods

Effects on bees:	Oral: LD_{50} > 222 µg MPCP/bee, only stated as µg Contact: LD_{50} > 185 µg MPCP/bee, only stated as µg
Risk assessment:	The risk to bees is assessed as negligible $LD_{50\ oral}$ > 4042 µg/MPCP/bee, only stated as µg HQ Oral < 15.8 HQ Contact < 18.9 (trigger value: 50)
Effects on terrestrial arthropods other than bees:	14.1 % mortality of *Typlodromus pyri* when dosed with 3.9 kg MPCA/ha, only stated as kg/ha
Risk assessment:	The risk to terrestrial arthropods is assessed as low

Effects on soil organisms

Effects on other terrestrial invertebrates:	NOEC ≥ 1000 mg MPCA/kg soil for earthworms, only stated as mg/kg soil
Risk assessment:	The risk to earthworms is assessed as low TER: 289 (trigger value: 10)
Effects on soil microorganisms:	NOEL ≥ 0.226 µL MPCP/10 g soils, only stated as µg/10 g soil
Risk assessment:	The risk to soil microorganisms is assessed as low

Additional studies

	An evaluation of the impact of aerial applications of commercial preparations of *B. thuringiensis* subsp. *kurstaki* against Spruce budworm in Canada on selected components of the environment (birds, small mammals, domestic honeybees, non-target insects, parasites of Spruce budworm and aquatic fauna) demonstrated that no adverse effects directly attributable to *B. thuringiensis* subsp. *kurstaki* on any of the monitored fauna occurred.

BACILLUS THURINGIENSIS KURSTAKI PB 54

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

Active micro-organism	*Bacillus thuringiensis* subsp. *kurstaki* strain PB-54
Function (*e.g.* fungicide)	Insecticide

Identity of the micro-organism (Annex IIM 1)

Name of the organism:	*Bacillus thuringiensis*
Taxonomy:	Bacillaceae; Bacillus
Species, subspecies, strain:	*Bacillus thuringiensis* subsp. *kurstaki* strain PB-54
Identification:	Confidential information, see Volume 4
Culture collection:	CECT 7209 (Spanish Collection of Cultures Types)
Minimum and maximum concentration of the micro-organism used for manufacturing of the formulated product (cfu/g; cfu/L, etc.):	5.09 x 10^9 CFU/g nominal concentration
Identity and content of relevant impurities in the technical grade micro-organism:	Open
Is the MCPA genetically modified; if so provide type of modification	No modification

Biological properties of the micro-organism (Annex IIM)

Origin and natural occurrence, background level:	*B. thuringiensis* subsp. *kurstaki* is indigenous and ubiquitous in many environment such as insects, soil and plant surfaces.
Target organism(s):	*Helicoverpa armigera*, Scare bordered straw (tomato fruitworm, old world bollworm, tomato grub, tobacco budworm, cotton bollworm, corn earworm)
Mode of action:	The crystal proteins of *B. thuringiensis* must be ingested to be effective against the target insect. Upon ingestion of *B. thuringiensis* by the larvae, the crystalline inclusions dissolve in the larval midgut, releasing insecticidal crystal proteins. The activated Cry toxins interact with the midgut epithelium cells of susceptible insects. After binding to the midgut receptors, they insert into the apical membrane to create ion channels, or pores, disturbing the osmotic balance and permeability. This can result in colloid-osmotic lysis of the cells. Spore germination and proliferation of vegetative cells into the haemocoel may result in septicaemia, contributing to mortality of the insect larvae.
Host specificity:	The strain PB-54 is highly specific against insect larvae of the order *Lepidoptera*

Life cycle:	Under beneficial conditions regarding moisture, temperature and nutrients, the basic metabolizing cell type is the vegetative cell that is actively growing and dividing. When a population of vegetative cells passes out of the exponential phase of growth, usually as a result of nutrient depletion, the differentiation of endospores begins. During sporulation, *B. thuringiensis* produces inclusion bodies that are composed of Cry proteins. An endospore will germinate and form a vegetative cell, when favourable conditions return for the growth of these cells. Before germination an endospore could pass through an extended period of dormancy, and is not able to replicate it self or form other spores.
Infectivity, dispersal and colonisation ability:	*B. thuringiensis* subsp. *kurstaki* strain PB-54 is specific to several species of insects of the order *Lepidoptera* and no cases of infectivity in other animal organisms or in plants is reported. *B. thuringiensis* subsp. *kurstaki* can be dispersed by the movement of insects and other animals carrying the bacteria in or on their bodies. The bacterium has poor colonization ability and is not a good competitor in the soil. Its survival is dependent on the presence and activity of other soil microorganisms and protection from degradation effects of sunlight. Applied as a spray on above ground leaves and fruits, endospores are rapidly inactivated and δ-endotoxins are rapidly degradable when exposed to UV-radiation.
Relationship to known pathogens:	*B. thuringiensis* is a member of the *Bacillus cereus* group which comprises closely related Gram positive bacteria that exhibit highly divergent pathogenic properties, including human pathogenic species, *B. cereus* and *Bacillus anthracis*. *B. cereus* can cause food poisoning by the production of toxins, whereas *B. anthracis* is the causative agent of anthrax.
Genetic stability:	Stable
Production of relevant metabolites/toxins:	Open
Resistance/sensitivity to antibiotics/anti-microbial agents used in human or veterinary medicine:	The strain PB-54 is resistant to Ampicillin, Cephalothin, Penicillium G, Trimethoprim-sulfamethoxazole. It is sensitive to Clindamycin, Erythromycin, Gentamicin, Nitrofurantoin, Oxacillin, Tetracycline and Vancomycin.

Classification and proposed labelling

with regard to the micro-organism:	Microorganism may have the potential to provoke sensitising reactions.

Summary of representative uses evaluated

Crop and/ or situation (a)	Member State or Country	Product name	F G or I (b)	Pests or Group of pests controlled (c)	Preparation		Application				Application rate per treatment			PHI (days) (m)	Remarks
					Type (d-f)	Conc. of as (i)	method kind (f-h)	growth stage & season (j)	number min/ max (k)	interval between applications (min)	kg as/hL; cfu/hL min – max (l)	water L/ha min – max	kg as/ha; cfu/ha min – max (l)		
Tomato (field and greenhouse use)	Europe	Belthirul	F/G	*Helicoverpa armigera* (Scarce bordered straw, tomato fruitworm, old world bollworm)	WP	160 g/kg 32,000 IU/mg 8.14 x 10^{11} CFU/kg	Foliar spraying	Any stage of growth	6-12	7 days	0.004-0.01	800-1000	0.04-0.08 2-4 x 10^{11} CFU/ha (0.25-0.5 kg product/ha)	No PHI required	-

* For uses where the column "Remarks" is marked in grey further consideration is necessary. Uses should be crossed out when the notifier no longer supports this use(s).
(a) For crops, the EU and Codex classifications (both) should be taken into account; where relevant, the use situation should be described (e.g. fumigation of a structure)
(b) Outdoor or field use (F), greenhouse 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/kg or cfu/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) The values should be given in g or kg whatever gives the more manageable number (e.g. 200 kg/ha instead of 200 000 g/ha or 12.5 g/ha instead of 0.0125 kg/ha) as well as in number of cfu
(m) PHI - minimum pre-harvest interval

Chapter 2 Analytical methods

Analytical methods for the micro-organism (Annex IIM 4.2, 4.3; IIIM 5.4)

Manufactured micro-organism (principle of method):	Open
Impurities and contaminating micro-organisms in manufactured material (principle of method):	Open
Microbial plant protection product (principle of method):	Open

Analytical methods for residues (viable and non-viable) (Annex IIM 4.5)

of the active micro-organism (principle of method):	Open
of relevant metabolites (principle of method):	Open

Chapter 3 Impact on Human and Animal Health (Annex IIM 5; IIIM 7)

Medical data, surveillance and observations:	Only in a few cases *Bt* has been isolated from human bacterial infection. One case of corneal ulcer after accidental splashing of a worker with *Btk* product. No evidence of adverse health effect among manufacturing plant personnel exposed to strain ABTS-351. No gastro-intestinal symptoms correlated with the presence of *Bt* in the faeces were observed in greenhouse workers spraying *Btk* PB-54. No major effects were observed in populations exposed to aerial spraying of *Btk* (upper airways and gastrointestinal symptoms) but no clear relationship could be established with *Btk.*
Sensitisation (experience in humans and study results; type of study):::	Potential risk of skin sensitization and induction of IgE and IgG antibodies in persons exposed to *B. thuringiensis kurstaki*. 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 eposure:	$LD_{50} > 1 \times 10^8$ CFU/rat

after acute inhalation exposure:	No study available with *Btk* PB-54. Based on data with other *Bt* species, intratracheal or intranasal administration of a high dose (10^8 CFU/animal) caused mortality but this was attributed to a mechanical/local effect not relevant for the human risk assessment. Lower doses applied intranasally (10^7 spores or less) induced local inflammation. After exposure by inhalation, no adverse effects were observed.
after acute intraperitoneal/subcutaneous exposure:	Intraperitoneal: NOAEL: 1 x 10^7 CFU/rat

Infectivity

after acute oral eposure:	Clearance not investigated. Based on data with other *Bt* species, *Btk* PB-54 is expected to have a slow clearance from the gastro-intestinal tract, and even to translocate to several organs, without causing pathogenicity. No further data are required.
after acute inhalation exposure:	Clearance not investigated. Based on data with other *Bt* species, *Btk* PB-54 is expected to have a slow clearance from the lungs without causing pathogenicity. No further data are required.
after acute intraperitoneal/subcutaneous exposure:	Clearance not investigated. Based on data with other *Bt* species, *Btk* PB-54 is expected to have a slow clearance after intraperitoneal administration, without causing pathogenicity. No further data are required.

Pathogenicity

after acute oral eposure:	No adverse effects observed
after acute inhalation exposure:	No study presented. Based on data for other *Bt* species, no adverse effects are expected after exposure by inhalation.
after acute intraperitoneal/subcutaneous exposure:	No adverse effects observed

Genotoxicity	No validated methods available for microorganisms. Potential toxins might need to be further investigated for their genotoxic properties.

Cell culture study:	No data available for *Btk* PB-54. Non-specific cytotoxicity of *Btk* ABTS-350 for 7 human cell types. Results not relevant for *Btk* (which are not intracellular replicating bacteria).

Short term toxicity/pathogenicity:	No studies available with *Btk* PB-54. Short term toxicity extrapolated from studies with *Btk* ABTS-351: 13-wk rat, oral: no adverse effect at a dose > 10^9 spores/animal/d; no data on infectivity/clearance 2-yr rat, oral: decreased body weight gain at a dose >10^{10} spores/animal/d; no data on infectivity/clearance 4-wk, guinea pigs, inhalation: NOAEL of 1.2 x 10^7 CFU/L based on lung lesions at 1.2 x 10^8 CFU/L; no data on infectivity/clearance
Specific toxicity, pathogenicity and infectiveness studies:	Potential toxins might need to be further investigated for their toxicological properties. Several acute toxicity studies were performed with the preparation (Belthirul).

Reference Values

AOEL	Not applicable. *Bacillus thuringiensis* strains cannot be excluded to produce enterotoxins (as *Bacillus cereus*) known to cause food poisoning in humans. In most instances, foodborne diseases caused by *Bacillus cereus* were associated with the intake of 10^5 to 10^8 CFU/g food. Lower numbers were reported in some outbreaks (10^3 to 10^4 CFU/g food).
ADI	Not applicable. *Bacillus thuringiensis* strains cannot be excluded to produce enterotoxins (as *Bacillus cereus*) known to cause food poisoning in humans. In most instances, foodborne diseases caused by *Bacillus cereus* were associated with the intake of 10^5 to 10^8 CFU/g food. Lower numbers were reported in some outbreaks (10^3 to 10^4 CFU/g food).

Exposure scenarios (including method of calculation)

Application method:	Spraying of tomatoes, in fields or greenhouses, tractor-mounted or hand-held.
Operator:	The exposure models for chemicals are not appropriate for micro-organisms. Exposure estimates with the German model (field use) and the Dutch Greenhouse model were provided by the RMS but are not relied on. Based on a qualitative assessment (potential for sensitisation), the need of personal protective equipment (including respiratory) is highlighted for the operators.

Workers:	Based on the potential of microorganisms for sensitisation, the need of personal protective equipment is highlighted for re-entry workers.
Bystanders:	Based on the potential of microorganisms for sensitisation, the risk cannot be concluded for the bystanders during field use (no personal protective equipment can be foreseen).

Chapter 4 Residues

Residues on Treated Products, Food and Feed (Annex IIM 6; IIIM 8)

Non-viable residues:	Open
Viable residues:	Open

Chapter 5 Fate and behaviour in the environment (Annex IIM 7; IIIM 9)

Persistence and multiplication

in soil:	No data on the persistence and multiplication of *Bacillus thuringiensis* subsp. *kurstaki* strains PB-54-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. PEC_{soil} Application rate: 0.5 kg product Belthirul/ha x 12 = 6 kg Belthirul/ ha (equivalent to 0.96kg Btk/ha). Incorporation in soil; 5 cm Soil density of 1.5 g/cm3 Soil interception 0 %. PEC_{soil}: 8.0 mg Belthirul/kg dry weight soil corresponding to 1.28 mg Btk/kg dry weight soil and 6.5 x 10^6 CFU/kg dry weight soil.
in water:	No data on the persistence and multiplication of *Bacillus thuringiensis* subsp. *kurstaki* strains SA-11, SA-12 and EG 2348 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} for ~~6~~ 12 applications of 0.5 kg Belthirul $PEC_{surface\ water}$: 160.24 µg Belthirul/L corresponding to 25.64 µg Btk/L and 1.3 x 10^5 CFU/L water.
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.

Chapter 6 Effects on Non-target Organisms (Annex IIM 8; IIIM 10)

Effects on terrestrial vertebrates

Risk assessment for mammals:	The risk to mammals is assessed as low.
Effects on birds:	No data were submitted on the strain PB54. Open literature do not indicate any risk of *B. thuringiensis* to birds.
Risk assessment:	The risk to birds is assessed as low . LD_{50} (Btk) > 740 mg/kg b.w. (minimum value) TER (insectivorous bird): > 306.70 TER (medium herbivorous bird): > 152.14
Effects on other terrestrial vertebrates:	Rat oral: $LD_{50} > 1 \times 10^8$ CFU/rat. No further studies provided.
Risk assessment:	The risk to terrestrial vertebrates is assessed as low.

Effects on aquatic organisms

Effects on fish:	No data were submitted on the strain PB54. Open literature do not indicate any risk of *B. thuringiensis kurstaki* strains to fish.
Risk assessment:	The risk to fish is assessed as low. LC_{50} (Bt) > 2.9×10^9 CFU/L (minimum value) TER: > 22308
Effects on freshwater invertebrates:	No data were submitted on the strain PB54. Open literature do not indicate any risk of *B. thuringiensis kurstaki* strains to freshwater invertebrates.
Risk assessment:	The risk to freshwater vertebrates is assessed as low . LC_{50} (Bt) > 600 IU/mL TER: > 117
Effects on algae:	No data were submitted on the strain PB54. Open literature do not indicate any risk of *B. thuringiensis kurstaki* strains to algae.
Risk assessment:	The risk to algae is assessed as low .
Effects on aquatic plants:	No data were submitted on the strain PB54. Open literature do not indicate any risk of *B. thuringiensis kurstaki* strains to aquatic plants.
Risk assessment:	The risk to aquatic plants is assessed as low .

Effects on arthropods

Effects on bees:	No data were submitted on the strain PB54. Open literature do not indicate any risk of *B. thuringiensis kurstaki* strains to bees.
Risk assessment:	The risk to bees is assessed as low.

	LD_{50} (Bt) > 100 µg/bee HQ < 9.6
Effects on terrestrial arthropods other than bees:	No data were submitted on the strain PB54. Open literature do not indicate any risk of *B. thuringiensis kurstaki* strains to terrestrial arthropods apart from non-target Lepidopteran species.
Risk assessment:	The risk to terrestrial arthropods is assessed as low.

Effects on soil organisms

Effects on other terrestrial invertebrates:	No data were submitted on the strain PB54. Open literature do not indicate any risk of *B. thuringiensis kurstaki* strains to earthworms.
Risk assessment:	The risk to terrestrial invertebrates is assessed as low . LC_{50} (other Btk product) > 6000 mg/m² (corresponding to 533 mg Belthirul/kg soil) TER: > 66.6
Effects on soil micro-organisms:	No data were submitted on the strain PB54. Open literature do not indicate any risk of *B. thuringiensis kurstaki* strains to soil micro-organisms.
Risk assessment:	The risk to soil microorganisms is assessed as low.

Additional studies

No further studies provided.

BACILLUS THURINGIENSIS KURSTAKI SA-11, SA 12, EG 2348

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

Active micro-organism	*Bacillus thuringiensis* subsp. *kurstaki* strains SA-11, SA-12 and EG2348
Function (*e.g.* fungicide)	Insecticide

Identity of the micro-organism (Annex IIM 1)

Name of the organism:	*Bacillus thuringiensis*
Taxonomy:	Bacillaceae: Bacillus
Species, subspecies, strain:	*Bacillus thuringiensis* subsp. *kurstaki* strains SA-11, SA-12 and EG2348
Identification:	Confidential information, see Volume 4
Culture collection:	SA-11 : NRRL B-30790 SA-12 : NRRL B-30791 EG2348 : NRRL B-18208
Minimum and maximum concentration of the micro-organism used for manufacturing of the formulated product (cfu/g; cfu/L, etc.):	4.85 x 10^{13} CFU/kg nominal concentration
Identity and content of relevant impurities in the technical grade micro-organism:	Open
Is the MCPA genetically modified; if so provide type of modification	No modification

Biological properties of the micro-organism (Annex IIM)

Origin and natural occurrence, background level:	SA-11 was re-isolated from the strain HD-500, which was originally derived from an insect. SA-12 was originally isolated from the almond moth (*Ephestia cantella*). EG2348 is a transconjugant, with HD269 as the host receiving a plasmid from subsp. *aizawai*. *B. thuringiensis* is ubiquitous in many environments such as insects, soil and plant surfaces.
Target organism(s):	European Grape Vine Moth (*Lobesia botrana*) and the Grape Bud Moth (*Eupoecelis ambiguella*)
Mode of action:	The crystal proteins of *B. thuringiensis* must be ingested to be effective against the target insect. Upon ingestion of *B. thuringiensis* by the larvae, the crystalline inclusions dissolve in the larval midgut, releasing insecticidal crystal proteins. The activated Cry toxins interact with the midgut epithelium cells of susceptible insects. After binding to the midgut receptors, they insert into the apical membrane to create ion channels, or pores, disturbing the osmotic balance and permeability. This can result in colloid-osmotic lysis of the cells. Spore germination and proliferation of vegetative cells into the haemocoel may result in septicaemia, contributing to the mortality of the insect larvae.
Host specificity:	The strains SA-11, SA-12 and EG2348 are highly specific against insect species of the order *Lepidoptera*.
Life cycle:	Under beneficial conditions regarding moisture, temperature and nutrients, the basic metabolizing cell type is the vegetative cell that is actively growing and dividing. When a population of vegetative cells passes out of the exponential phase of growth, usually as a result of nutrient depletion, the differentiation of endospores begins. During sporulation, *B. thuringiensis* produces inclusion bodies that are composed of Cry proteins. Endospores will germinate and form vegetative cells, when favourable conditions return for the growth of these cells. Before germination an endospore could pass through an extended period of dormancy, and is not able to replicate itself or form other spores.

Infectivity, dispersal and colonisation ability:	*B. thuringiensis* subsp. *kurstaki* strains SA-11, SA-12 and EG2348 are specific to several species of insects of the order *Lepidoptera* and no cases of infectivity in other animal organisms or in plants are reported. *B. thuringiensis* subsp. *kurstaki* can be dispersed by the movement of insects and other animals carrying the bacteria in or on their bodies. The bacterium has poor colonization ability and is not a good competitor in the soil. Its survival is dependent on the presence and activity of other soil microorganisms and protection from degradation effects of sunlight. Applied as a spray on above ground leaves and fruits, endospores are rapidly inactivated and δ-endotoxins are rapidly degradable when exposed to UV-radiation.
Relationship to known pathogens:	*B. thuringiensis* is a member of the *Bacillus cereus* group which comprises closely related Gram positive bacteria that exhibit highly divergent pathogenic properties, including two human pathogenic species, *B. cereus* and *Bacillus anthracis*. *B. cereus* can cause food poisoning by the production of toxins, whereas *B. anthracis* is the causative agent of anthrax.
Genetic stability:	Stable
Production of relevant metabolites/toxins:	Open
Resistance/sensitivity to antibiotics/anti-microbial agents used in human or veterinary medicine:	SA-11, SA-12 and EG2348 are intrinsic resistant to Penicillin G. SA-11 and SA-12 are sensitive to Amikacin, Chloramphenicol, Clindamycin, Doxycycline, Erythromycin, Gentamicin, Kanamycin, netilmicin, Nitrofurantoin, Sulfisoxazole, Streptomycin, Tetracycline, Tobramycin, Trimethoprim-sulfamethoxazole and Vncomycin. EG2348 is susceptible to Erythromycin, Chloramphenicol, Streptomycin and Clindamycin.

Classification and proposed labelling

with regard to the micro-organism:	Microorganisms may have the potential to provoke sensitising reactions:

Summary of representative uses evaluated

Crop and/ or situation (a)	Member State or Country	Product name	F G or I (b)	Pests or Group of pests controlled (c)	Formulation		Application				Application rate per treatment			PHI (days) (l)	Remarks (m)
					Type (d-f)	Conc. of MPCA (i)	Method Kind (f-h)	Growth stage & season (j)	Number min max (k)	Interval between applications (min)	Kg MPCA/hL min max	water L/ha min max	Kg MPCA/ha CFU MPCA/ha min max		
Grapes	Northern and southern Europe	Delfin WG	F	European Grape Vine Moth (*Lobesia botrana)* European Grape Berry Moth (*Eupoecelis ambiguella)*	WG	85 % 4.85 x 10^{13} CFU/kg product Potency: 32,000 IU/mg 850g spores per kg product*	Spraying	From bud break up to harvest (BBCH 53-99)	1-3	7 days	0.06-0.32	400 – 1500	0.85-1.275 4.85-7.3 x 10^{13} CFU/ha (1.0 - 1.5 kg/ha product)	No PHI required	

(a) For crops, the EU and codex classifications (both) should be used; where relevant, the 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 sucking insects, soil born insects, foliar fungi, weeds
(d) e.g. wettable powder (WP), emusifiable concentrate (EC), granule (GR)
(e) GCPF codes – Crop Life Technical Monograph No 2, 1989
(f) All abbreviations used must be explained
(g) Method, e.g. high 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
* *Bacillus thuringiensis* ssp. *kurstaki* spores, solids and lepidopteran active toxins

Chapter 2 Analytical methods

Analytical methods for the micro-organism (Annex IIM 4.2, 4.3; IIIM 5.4)

Manufactured micro-organism (principle of method):	Open
Impurities and contaminating micro-organisms in manufactured material (principle of method):	Open
Microbial plant protection product (principle of method):	Open

Analytical methods for residues (viable and non-viable) (Annex IIM 4.5)

of the active micro-organism (principle of method):	Open
of relevant metabolites (principle of method):	Open

Chapter 3 Impact on Human and Animal Health (Annex IIM 5; IIIM 7)

Medical data, surveillance and observations:	Only in a few cases *Bt* has been isolated from human bacterial infection. One case of corneal ulcer after accidental splashing of a worker with *Btk* product. No evidence of adverse health effect among manufacturing plant personnel exposed to the strains SA-11/SA-12/EG-2348. No major effects were observed in populations exposed to aerial spraying of *Btk* (upper airways and gastrointestinal symptoms) but no clear relationship could be established with *Btk.*
Sensitisation (experience in humans and study results; type of study):	Potential risk of skin sensitization and induction of IgE and IgG antibodies in persons exposed to *B. thuringiensis kurstaki*. 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} > 4.6 x10^{11} CFU of SA-12 /kg bw
after acute inhalation exposure:	Inhalation study (4h): rat LC_{50} > 3.4 x 10^{7} CFU of SA-11/L
after acute intravenous exposure:	Rat NOAEL = 1.2 x 10^{8} CFU of SA-11/kg bw
Infectivity	
after acute oral exposure:	Clearance not investigated. Based on data with other *Bt* species and on short term toxicity studies, *Btk* SA-11/SA-12/EG-2348 are expected to have a slow clearance from the gastro-intestinal tract, and even to translocate to several organs, without causing pathogenicity. No further data are required.
after acute inhalation exposure:	Inhalation study: high numbers of bacteria in lung and caecum at day 22. Smaller numbers in lymph nodes, liver and spleen at day 22.
after acute intravenous exposure:	Persistent high numbers of bacteria in lymph nodes, spleen and liver throughout the 21-day observation period.
Pathogenicity	

after acute oral exposure:	Transient gastrointestinal symptoms (oily anal discharge, diarrhoea, decreased defecation). Discolouration of liver or kidney in 4 out of 5 male rats.
after acute inhalation exposure:	Wet fur and changes in respiratory pattern. Based on data with other *Bt* species, intranasal administration of a high dose (10^8 CFU/animal) caused mortality but this was attributed to a mechanical/local effect not relevant for the human risk assessment. Lower doses applied intranasally (10^7 spores or less) induced local inflammation.
after acute intravenous exposure:	No adverse effects.
Genotoxicity:	No validated methods available for microorganisms. Potential toxins might need to be further investigated for their genotoxic properties.
Cell culture study:	Non-specific cytotoxicity of *Btk* ABTS-351 for 7 human cell types. Results not relevant for *Btk* (which are not intracellular replicating bacteria).
Short term toxicity/pathogenicity:	13-wk rat, oral, SA-11: NOAEL = 10^8 CFU/rat; CFU found in the lungs and caecum, complete clearance after recovery period in the caecum, lower numbers but incomplete clearance in the lungs. Short term toxicity by inhalation extrapolated from studies with Btk ABTS-351: 4-wk, guinea pigs, inhalation: NOAEL of 1.2×10^7 CFU/L based on lung lesions at 1.2×10^8 CFU/L; no data on infectivity/clearance
Specific toxicity, pathogenicity and infectiveness studies:	SA-12: Well-defined to severe erythema in dermal toxicity study (rat). Slightly irritating to the skin and eye of rabbits. SA-11: mouse oral $LD_{50} > 36 \times 10^{10}$ CFU/kg bw, no significant increase of micronuclei in bone marrow polychromatic erythrocytes.

AOEL	Not applicable *Bacillus thuringiensis* strains cannot be excluded to produce enterotoxins (as *Bacillus cereus*) known to cause food poisoning in humans. In most instances, foodborne diseases caused by *Bacillus cereus* were associated with the intake of 10^5 to 10^8 CFU/g food. Lower numbers were reported in some outbreaks (10^3 to 10^4 CFU/g food).
ADI	Not applicable *Bacillus thuringiensis* strains cannot be excluded to produce enterotoxins (as *Bacillus cereus*) known to cause food poisoning in humans. In most instances, foodborne diseases caused by *Bacillus cereus* were associated with the intake of 10^5 to 10^8 CFU/g food. Lower numbers were reported in some outbreaks (10^3 to 10^4 CFU/g food).

Exposure scenarios (German BBA model)

Application method:	Spraying of grapevines, in fields
Operator:	The exposure models for chemicals are not appropriate for micro-organisms. Based on a qualitative assessment (potential for sensitisation), the need of personal protective equipment (including respiratory) is highlighted for the operators.
Workers:	Based on the potential of microorganisms for sensitisation, the need of personal protective equipment is highlighted for re-entry workers.
Bystanders:	Based on the potential of microorganisms for sensitisation, the risk cannot be concluded for the bystanders during field use (no personal protective equipment can be foreseen).

Chapter 4 Residues

Residues on Treated Products, Food and Feed (Annex IIM 6; IIIM 8)

Non-viable residues:	Open
Viable residues:	Open

Chapter 5 Fate and behaviour in the environment (Annex IIM 7; IIIM 9)

Persistence and multiplication

in soil:	No data on the persistence and multiplication of *Bacillus thuringiensis* subsp. *kurstaki* strains SA-11, SA-12 and EG 2348 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. PEC_{soil} Application rate: 1.5 kg product Delfin WG/ha x 3= 4.5 kg Delfin WG / ha Incorporation in soil; 5 cm Soil density of 1.5 g/cm3 Soil interception 0 %. PEC_{soil}: 6 mg Delfin WG/kg dry weight soil (5.1 mg MPCA/kg dry weight soil). In terms of CFU, this is equivalent to 2.91 x 10^5 CFU/g dry weight soil.
in water:	No data on the persistence and multiplication of *Bacillus thuringiensis* subsp. *kurstaki* strains SA-11, SA-12 and EG 2348 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. According to the PEC calculation, the predicted initial concentration of Delfin WG in surface water following one application of 1.5 kg a.s. / ha (spray drift 8 %) is 46.56 µg Delfin WG/L (39.57 µg MPCA/L). In terms of CFU, this is equivalent to 2.25 x 10^6 CFU/L (40.06 µg Delfin WG/L x 4.85 x 10^4 CFU/µg). When a maximum of three applications are considered (1.5 kg a.s /ha x 3= 4.5 kg a.s. / ha; spray drift 6.90 %) and no degradation

	over the intervening 14 days between the first and third applications is assumed, the predicted initial concentration of Delfin WG in surface water is 103.40 µg Delfin WG/L (87.89 µg MPCA /L). In terms of CFU, this is equivalent to 5.02 x 10^6 CFU/L (103.40 µg Delfin WG/L x 4.85 x 10^4 CFU/µg).
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.

Chapter 6 Effects on Non-target Organisms (Annex IIM 8; IIIM 10)

Effects on terrestrial vertebrates

Risk assessment for mammals:	The risk to mammals is assessed as low
Effects on birds:	Northern bobwhite: LD_{50} > 3333 mg MPCA/kg b.w. only stated as mg/kg
Risk assessment:	The risk to birds is assessed as low.
Effects on other terrestrial vertebrates:	Rat oral: LD_{50}> 5050 MPCP mg/kg b.w (2×10^{11} CFU/kg b.w.)
Risk assessment:	The risk to terrestrial vertebrates is assessed as low.

Effects on aquatic organisms

Effects on fish:	Rainbow trout: LC_{50}> 5.3×10^{8} CFU MPCA/L (5.3 mg /L) only stated as mg/L
Risk assessment:	TER = 60.3 (based on PECsw of 87.89 µg MPCA /L). High risk to fish not excluded based on the TER (trigger 100), but overall considered low based on a weight of evidence approach.
Effects on freshwater invertebrates:	*Daphnia magna*: 21-day semi static EC_{50}> 8.4×10^{8} CFU/L (41.5 mg MPCA/L) TER= 472 (based on PECsw of 87.89 µg MPCA /L)
Risk assessment:	The risk to freshwater invertebrates is assessed as low.
Effects on algae:	*Selenastrum capricornotum*: 72 hour EC_{50}> 1.0×10^{6} CFU/L (42 mg MPCA /L) *Selenastrum capricornotum*: 96 hour EC_{50}> 1.47 mg MPCA/L (oil based flowable formulation) TER = 477 (based on PECsw of 87.89 µg MPCA /L)
Risk assessment:	The risk to algae is assessed as low.
Effects on aquatic plants:	No studies submitted
Risk assessment:	The risk to aquatic plants is assessed as negligible.

Effects on arthropods

Effects on bees:	*Apis millifera*: LD_{50}>25 µg MPCA/bee only stated as µg
Risk assessment:	The risk to bees is assessed as low.
Effects on terrestrial arthropods other than bees:	*Aphidius rhopalosiphi*:LR_{50}> 4.5 kg Delfin/ha ,only stated as kg /ha Mortality: 12.5% Reduction of reproduction rate: 19% *Typhlodromus pyri*: LR_{50}> 4.5 kg Delfin/ha ,only stated as kg /ha

	Mortality: 12 % Reduction of reproduction rate: - 44% (increase in reproduction rate) *Brachymeria intermedia*:LR_{50}> 0.56 mg MPCA /larvae .only stated as mg/larvae Mortality: 15% *Chrysoperla rufilabris*:LR_{50}> 0.56 mg MPCA /larvae only stated as mg/larvae Mortality: 0 *Chrysoperla carnea*:LD_{50}> 2.24 kg MPCA /ha ,only stated as kg/ha Mortality: 4%
Risk assessment:	The risk to terrestrial arthropods is assessed as low.

Effects on soil organisms

Effects on other terrestrial invertebrates:	*Eisenia fetida*:LC_{50}>1000 mg Delfin/kg soil d.w. only stated as in mg Mortality: 2.5% Increased bodyweight
Risk assessment:	The risk to terrestrial invertebrates is assessed as low.
Effects on soil micro-organisms:	Delfin WG has no effect (less than 25%) on the nitrogen turnover and short-term respiration activity of soil micro-flora after 42 days at tested concentrations of up to 20 mg/kg soil.
Risk assessment:	The risk to soil microorganisms is assessed as low.

APPENDIX B – USED COMPOUND CODE(S)

Code/Trivial name*	Chemical name**	Structural formula**
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* The metabolite name in bold is the name used in the conclusion.

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
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
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