SECTION IIIA 7	**FATE AND BEHAVIOUR IN THE ENVIRONMENT**	**Official use only**
IIIA 7.1	**Persistence and multiplication**	
IIIA 7.1.1 Soil	*Bti* is not infectious and survives poorly in the environment resulting in a limited spread of the organism. Vegetative cells of *Bti* have a limited survival time in the environment and spores do not germinate readily, making it highly unlikely that *Bti* will multiply and colonise areas of intended use above levels that may occur naturally. *Bti* is not persistent in soil and water and airborne concentrations of the organism are expected to be negligible following application to water bodies. Further information is contained in a review by Glare and O'Callaghan and in the following study summaries.	
IIIA 7.1.1-01	**Soil**	
Reference	Goodyear, A. (2005). Behaviour of the Microbial Pest Control Agent *Bacillus thuringiensis* subsp *israelensis* in Soil. TSGE unpublished report number 22-1-05.SOIL.	
Data protection	Yes	
Data owner	Valent BioSciences	
Companies with letter of access	None	
Criteria for data protection	Submitted on an existing a.s. for the purpose of entry into Annex I.	
Guideline study	Not applicable (review of published literature)	
GLP	No (review of published literature)	
Deviations	None	
Materials and Methods	Information relating to the behaviour of *Bti* (Strain AM65-52) in soil are summarised in terms of the methodology used and the results and conclusions relevant to the behaviour of *Bti* (Strain AM65-52) in soil. Where studies have been performed on Bt subspecies other than *israelensis* the results are considered to be representative of the likely behaviour of *israelensis* (*Bti* (Strain AM65-52)) under the conditions of the test.	
Results	Petras, S.F. and Casida, L.E. Survival of *Bacillus thuringiensis* Spores in Soil. Applied and Environmental Microbiology. Dec 1985. p 1496 – 1501. The behaviour of *Bacillus thuringiensis* in soil was investigated using vegetative cells, spores and crystal toxins from the *Bacillus thuringiensis* (H-type 3a3b-2) serovar. The survival of the bacterium in soil under laboratory and field conditions was studied and included the effects of soil pH, nutrient amendment and soil drying. The effect of spore and crystal toxin addition on the number of soil organisms present was also observed. Experimental details are described in the report. *Bacillus thuringiensis* applied at a rate of *ca* 1 x 10^7 spores/g soil to three field sites showed a decrease in spore count of between 0.8 and 1.3 log during the first 2 weeks of the study. Thereafter, the number of spores present remained roughly constant until the end of the study at 6 weeks. In the laboratory, *Bacillus thuringiensis* was incubated in sterile and non-sterile soil at both 4°C and 27°C over a period of 8 months and little change in spore count was observed under each of these conditions beyond the initial decline observed in the field. In	

SECTION IIIA 7	FATE AND BEHAVIOUR IN THE ENVIRONMENT	Official use only
IIIA 7.1	**Persistence and multiplication**	
	both the laboratory and field soils, a slightly greater initial spore death was observed in soils below pH 5.0. Soils incubated in the laboratory at 27°C and differing moisture levels (40, 60 and 80% MWHC) for a period of 8 weeks showed little change in survival. The only significant effect observed was a slight decease in spore viability where dry soil was remoistening. Heat treatment of spores (80°C for 20 minutes) had no effect on spore survival and the effect of heat did not induce germination of the spores. No effect on spore counts was observed in soils amended with either L-alanine-glucose, magnesium chloride, calcium chloride or manganese chloride; however amendment with HI and tryptone broth produced an initial decline in spores during the first 10 days incubation, followed by an increase over the next 20 days. This effect was further investigated and was shown to be due to a proportion of the spores germinating and re-sporulating. However, spores that were formed in-situ died and by 14 days the spores present were those that had not germinated originally. The effect of spore and crystal toxin addition on the number of soil organisms present is summarized in Table IIIA 8.9-01. Spores were added at a rate of 2×10^7 spores/g soil and incubated for 2 weeks at 27°C. No reduction in the numbers of each species counted was recorded, and for the majority of species a moderate increase was observed. *Bacillus thuringiensis* spores added to soil under natural and laboratory conditions show an initial decline in numbers equivalent to approximately one order of magnitude over a period of 2 weeks. Germination of spores was not effected by soil pH, by soil moisture, or by pre-treatment with heat. The addition of spores to soils did not produce a detrimental effect on the numbers of indigenous soil organisms over a 14 day period. West, A.W. *et al*. Persistence of *Bacillus thuringiensis* Parasporal Crystal Insecticidal Activity in Soil. Journal of Invertebrate Pathology. 44, 128-133 (1984). The persistence of the insecticidal parasporal crystal toxin was investigated in a clay loam soil (pH 5.0, organic carbon 2.3%) using an isolate of streptomycin-resistant *Bacillus thuringiensis* subsp. *aizawai* (serotype H-7, Strain HD137). Portions of non-sterile and sterile soil were inoculated with bacteria at a level of 10^9 spores/g soil and incubated in the dark at 25°C over a period of 842 days. Bacteria were extracted with peptone water and analysed by bioassay against *P.brassicae* larvae and by plating. Experimental details are described in the report. In non-sterile soil, applied *Bacillus thuringiensis* and indigenous bacteria declined exponentially with estimated half-lives of 68 and 375 days, respectively. In sterile soil, applied *Bacillus thuringiensis* bacteria declined exponentially with an estimated half-life of 300 days. In natural soil, the parasporal crystal remained fully active for 3 days before declining with a predicted loss of 77% activity after 100 days and 92% activity after 1000 days. No germination or growth of *Bacillus thuringiensis* spores was observed in either sterile or non-sterile soils and consequently no additional parasporal crystal were produced during incubation.	

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IIIA 7.1	**Persistence and multiplication**	
	Bacillus thuringiensis bacteria are not persistent in soil and decline under non-sterile conditions with an estimated half-life of 68 days. The degradation rate for the bacteria measured in sterile soil is substantially longer (300 days) suggesting that disappearance of the bacteria is mediated by indigenous soil micro-organisms. The parasporal crystal insect toxins are also rapidly degraded in non-sterile soil with *ca* 23% activity remaining after 100 day. The spores of *Bacillus thuringiensis* are more persistent in soil but do not germinate readily. Akiba, Y. Assessment of Rainwater-Mediated Dispersion of Field-Sprayed *Bacillus thuringiensis* in the Soil. Appl. Ent. Zool. 26 (4): 477-483 (1991). The level and movement of *Bacillus thuringiensis* in natural soils was investigated in relation to the amount of rainfall and artificial irrigation applied. The experiment was performed using *Bacillus thuringiensis* serovar *sotto* (H- antigen 4a:4b) Strain AF101 applied to different field plots containing volcanic clay loam, alluvium sand, alluvium loam and sand soils. Application was made at a rate of 1×10^{11} to 1×10^{12} spores/m^2 and samples were taken weekly for up to 5 weeks after treatment and at various depths up to 30 cm. All samples were analysed for viable *Bacillus thuringiensis* cell count by plating and for identity by crystal morphology. Leaching of *Bacillus thuringiensis* in soil columns was also investigated using samples of the treated field soil. Experimental details are described in the report. The dispersal of field sprayed *Bacillus thuringiensis* in the top 30 cm soil layer was assessed under naturally and artificially irrigated conditions. Following approximately one month of continuous rainfall during the summer rainy season in Kumagaya, Japan, *Bacillus thuringiensis* was only detected at the soil surface (0-1 cm depth) in two field plots and a reduction in the number of *Bacillus thuringiensis* cells of 71-99% occurred in the first week of a 34-day post application observation period. Following an artificial irrigation event over a two week period in the field, equivalent to 450 mm rainfall, *Bacillus thuringiensis* bacteria were only detected to a depth of 6 cm in the soil and at considerably lower concentrations than at the surface (approximate reduction in density of 1/1000). The vertical distribution and decline of *Bacillus thuringiensis* in two field plots (A and B) irrigated naturally is shown in Table IIIA 8.9-02. In both plots, *Bacillus thuringiensis* was detected only in the surface layer (0 to 1 cm) for the duration of the study. The number of *Bacillus thuringiensis* cells detected in the surface layer declined from 25.3×10^3 to 7.3×10^3 cells/g in soil A, and from 8.2×10^3 to 0.08×10^3 cells/g in soil B during the first week. Thereafter the number of *Bacillus thuringiensis* cells/g remained roughly constant. Similar results were obtained from two field plots (C and D) irrigated artificially with high levels of water as shown in Table IIIA 8.9-03. In laboratory experiments conducted with soil columns, *Bacillus thuringiensis* cells present at between 2.4×10^5 and 5.0×10^5 cells/g in soil were not present in the leachate water from columns containing a loam soil and were only present at low levels (up to 3.5×10^2 cells/mL) in leachate from columns containing a sand soil. *Bacillus thuringiensis* cells applied to field soils under natural	

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IIIA 7.1	**Persistence and multiplication**	
	conditions do not move appreciably through the soil profile. The lack of mobility was attributed to adsorption onto clay minerals and silica, whilst the disappearance of *Bacillus thuringiensis* in soil was considered to be the result of the inability of spores to germinate and a gradual de-activation by competitive soil micro-organisms and acidity in the soil. The results of this study clearly demonstrate that even after significant rainfall *Bacillus thuringiensis* does not penetrate deeply into soil. The potential for movement to groundwater is therefore considered to be extremely minimal. Venkateswerlu, G. and Stotsky, G. Binding of the Protoxin and Toxin Proteins of *Bacillus thuringiensis* subsp. *kurstaki* on Clay Minerals. Current Microbiology. Vol. 25 (1992), pp. 225-233. The sorption and binding of *Bacillus thuringiensis* delta-endotoxin proteins to clay fractions was studied using protoxin (132 kDa) and toxin (66 kDa) material isolated from *Bacillus thuringiensis* subsp. *kurstaki*. Solutions of isolated toxins were equilibrated with 0.2% sodium carbonate containing 100 to 500 µg of montmorillonite or kaolinite clay at room temperature. After equilibration, the mixtures were centrifuged and the amount of toxin present determined by UV_{280nm} absorbance. Desorption tests were performed using fresh portions of 0.2% sodium carbonate which were similarly analysed by UV_{280nm} absorbance. Bioassay for insecticidal activity with third instar larvae of *Trichoplusia ni* were also performed. Experimental details are described in the report. Equilibrium adsorption of both protoxin and toxin from *Bacillus thuringiensis* subsp. *kurstaki* on clay minerals occurred within 30 minutes. The maximum amount of protoxin adsorbed was equivalent to 22% for montmorillonite and 4.5% for kaolinite, with the corresponding values for the toxin equal to 38 and 13.5%, respectively. Adsorption of the proteins was concentration dependent and decreased by a factor of approximately 2 with an increase in pH from 4 to 10. No significant temperature effects were observed between 7 and 50°C. Almost complete (>90%) desorption of toxin and protoxin from kaolinite was possible using two or three washings with water and 0.2% sodium carbonate, however, corresponding treatment of the montmorillonite resulted in no desorption of toxin and protoxin. *Bacillus thuringiensis* insecticidal toxins are rapidly bound to clay particles. Adsorption is greater and less reversible for montmorillonite clay compared to kaolinite clay. Adsorption was pH dependent with lower adsorption observed at high pH, but was not affected by temperature. West, A.W. Survival of *Bacillus thuringiensis* and *Bacillus cereus* Spore Inocula in Soil: Effects of pH, Moisture, Nutrient Availability and Indigenous Microorganisms. Soil Biol. Biochem. Vol. 17, No. 5 pp. 657-665, 1985. The effect soil pH, moisture, nutrient availability and indigenous microorganism content on the growth of *Bacillus thuringiensis* subsp. *aizawai* (induced streptomycin resistant strain HD137) was studied using an agricultural sandy silt loam soil (Pulborough, West Sussex, UK). The soil was air dried and sieved (< 2 mm) prior to use. Portions (5 g) of the soil were placed in vials, inoculated with *Bacillus thuringiensis* subsp. *aizawai* spores at a level of 10^4 spores/g (dry weight) and incubated at 25°C in the dark. The soil conditions were	

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IIIA 7.1	**Persistence and multiplication**	
	varied by using combinations of the factors shown in Table IIIA 8.9-04. Soil samples were removed periodically and extracts (0.1% peptone water) were plated onto selective media. Bacillus colonies were examined microscopically and by gel electrophoresis to confirm the identity of the original bacteria. The experiment was run concurrently with *Bacillus cereus* bacteria however data generated with this bacterium are not relevant to this dossier and are not discussed in this summary. After inoculation into natural soil, numbers of *Bacillus thuringiensis* remained stable in all soil treatments except moisture saturated (0 MPa) soil at pH 5.2 where a slow increase was observed and the driest soil (-1.0 MPa) at pH 5.2 where a slow decrease was observed during incubation. After inoculation into autoclaved sterile soil, two distinct phases of survival were observed. During the first 5 days after incubation, populations of *Bacillus thuringiensis* increased in all soil treatments with the increase higher at pH 7.3 and more pronounced in drier soils. After 5 days the rate of increase slowed, especially in drier soils which had been adjusted to pH 7.3. In unamended sterile soils a decline in *Bacillus thuringiensis* population was observed after 5 days. During the first 3 hours incubation in nutrient supplemented natural soil, populations of *Bacillus thuringiensis* at pH 5.2 remained stable but declined sharply at pH 7.3. This initial phase was followed by a very rapid increase in populations with the highest increases observed in the driest soils and the lowest rates observed in soils at pH 7.3. The growth phase ended after 7 to 12 hours, after which time populations remained stable or gradually declined, with the exception of the driest soils where a slow increase continued. After inoculation into autoclaved sterile soil supplemented with nutrients, the survival of *Bacillus thuringiensis* was comparable to the results obtained from the natural soils supplemented with nutrients, with the exception that differences observed between moisture and pH were less pronounced. In natural soils growth of *Bacillus thuringiensis* was poor. Growth was improved by the removal of indigenous species (by sterilisation) and this was attributed to the removal of nutrient competition, a conclusion supported by the continued growth of *Bacillus thuringiensis* in nutrient supplemented soils. Raising the soil pH from 5.2 to 7.3 increased the rate of *Bacillus thuringiensis* growth in soils possibly as a result of a change in nutrient solubility and availability. An exception to this was observed in natural soils supplemented with nutrients where rates of *Bacillus thuringiensis* growth decreased, probably as a result of stimulation of indigenous species and increased nutrient competition. A pH threshold for *Bacillus thuringiensis* growth of between pH5.0 and pH 5.2 was suggested and was in general agreement with other work referenced in the report. Soil moisture was shown to be an important factor controlling populations of *Bacillus thuringiensis* with growth retarded in dry soils and stimulated in wet soils. Akiba, Y. Microbial Ecology of *Bacillus thuringiensis* VI. Germination of *Bacillus thuringiensis* Spores in the Soil. Appl. Ent. Zool. 21 (1): 76-80 (1986).	

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IIIA 7.1	**Persistence and multiplication**	
	The possibility that *Bacillus thuringiensis* spores may germinate in natural soils was investigated using *thuringiensis* and *morrisoni* subspecies of the bacterium. Three agricultural soil types (clay loam/clay, sand and sandy loam/loam) were inoculated with either spores or vegetative cells of the bacteria at a rate of 10^5 to 10^6 cells/g of soil and incubated at 30°C. Sub-samples were removed at periods of 0, 1, 2, 7 and 14 days after inoculation, extracted with distilled water and plated for total viable cells (vegetative and spores) on PP media and for vegetative cells only on BTV media. The study was performed with sterile and non-sterile soils. In non-sterile soils treated with spores, the total count of viable cells (vegetative and spores) of *Bacillus thuringiensis* subsp. *thuringiensis* decreased from 10^5 cells/g to 10^4 cells/g over a period of 14 days. The corresponding total cell count for the *morrisoni* subspecies was stable over the same period. On plating media selective for vegetative cells only no colonies were detected which showed that *Bacillus thuringiensis* spores did not germinate in live soils. In sterile soils, spores of *Bacillus thuringiensis* subsp. *thuringiensis* and *morrisoni* both germinated with subsequent vegetative growth during the test period, although the rate of germination and growth were different for the three soil types used. In non-sterile soils treated with vegetative cells, the total count of viable cells (vegetative and spores) of *Bacillus thuringiensis* subsp. *thuringiensis* decreased from 10^6 cells/g to 3-5 x 10^4 cells/g within 2 days and remained at a density of 10^4 cells/g for the rest of the incubation period. Vegetative cells only were detected at a level of 10^6 cells/g at the start of the test, but there were no vegetative cells present after 2 days incubation. In natural soils *Bacillus thuringiensis* remain in its spore state but is not able to germinate. Vegetative cells of *Bacillus thuringiensis* applied to soil are rapidly inactivated and disappear within 1 to 2 days. Further information on the fate of Bacillus thuringiensis in soil is given in the book by Glare and O'Callaghan[1]. In this reference, a half-life of 100 to 200 days is suggested for Bacillus thuringiensis spores, whilst the degradation of the Bacillus thuringiensis insecticidal crystal toxins is reported to be much more rapid with a half-life of 9.5 to 8.5 days given for grass and manure amended soils, respectively. Further information A half-life of 100 to 200 days is suggested for *Bacillus thuringiensis* spores, whilst the degradation of the *Bacillus thuringiensis* insecticidal crystal toxins is reported to be much more rapid with a half-life of 9.5 to 8.5 days given for grass and manure amended soils, respectively. Vegetative cells of *Bacillus thuringiensis* are also reported to be extremely sensitive to UV light, with survival reduce to 0.1% following exposure for 40 minutes. Slow germination and sensitivity to UV light are cited as reasons for a lack of persistence of *Bacillus thuringiensis* in soil.	
Applicant's Summary and	In natural soils *Bti* will exist predominantly in its spore form and	

1 Glare, T.R. and O'Callaghan, M. 2000. *Bacillus thuringiensis*: Biology, Ecology and Safety, John Wiley and Sons Ltd. p 71-79.

SECTION IIIA 7	FATE AND BEHAVIOUR IN THE ENVIRONMENT	Official use only
IIIA 7.1	**Persistence and multiplication**	
conclusion	spores may persist in soil for periods of 100 to 200 days. The spores do not germinate readily in soil and germination is not influenced by soil pH, soil moisture, or by pre-treatment of the spores with heat. In contrast, the vegetative cells of *Bacillus thuringiensis* bacteria are not persistent in soil and decline rapidly under non-sterile conditions due to competition from other indigenous soil bacteria and the effects of UV light. The survival time for vegetative cells in soil may be as short as 1 to 2 days, with growth reported to be slowed in dry conditions. The parasporal crystal toxins produced by *Bacillus thuringiensis* are also rapidly degraded in natural soils with a half-life in the order of 9 days reported in manure supplemented soil. *Bacillus thuringiensis* cells applied to field soils under natural conditions did not move appreciably through the soil profile. The lack of mobility was attributed to adsorption onto clay minerals and silica. *Bacillus thuringiensis* parasporal crystal toxins are also rapidly bound to clay particles and will be similarly non-mobile in soil. Rapid degradation of *Bti* vegetative cells and insecticidal toxins in soil and poor germination of *Bti* spores in soil show that the organism is not persistent and will not multiply in the soil environment. The little vertical dispersal that was seen has been attributed to factors such as water movement, the growth of plant roots and the actions of soil invertebrates such as earthworms[2] In the study by Akiba (1991) described above, the dispersal of field sprayed *Bacillus thuringiensis* in the top 30 cm soil layer was assessed under naturally and artificially irrigated conditions. Following approximately one month of continuous rainfall during the summer rainy season in Kumagaya, Japan, *Bacillus thuringiensis* was only detected at the soil surface (0-1 cm depth) in two field plots and a reduction in the number of *Bacillus thuringiensis* cells of 71-99% occurred in the first week of a 34-day post application observation period. Following an artificial irrigation event over a two week period in the field, equivalent to 450 mm rainfall, *Bacillus thuringiensis* bacteria were only detected to a depth of 6 cm in the soil and at considerably lower concentrations than at the surface (approximate reduction in density of 1/1000). The results of this study clearly demonstrate that even after significant rainfall *Bacillus thuringiensis* does not penetrate deeply into soil. The potential for movement to groundwater is therefore considered to be extremely minimal. Rapid degradation of *Bti* vegetative cells and insecticidal toxins in soil and poor germination of *Bti* spores in soil show that the organism is not persistent and will not multiply in the soil environment. Although *Bacillus thuringiensis* bacteria constitute an indigenous part of the soil micro-flora community, they do not compete aggressively with other soil micro-organisms and are not adapted to survive as an active member of the soil microbial community. The inability or low capacity of *Bacillus thuringiensis* spores to germinate in soil restricts population growth and no epizootics with *Bacillus thuringiensis* subsp. *israelensis* have ever been reported.	

[2] Hendriksen and Hansen. Long Term Survival and Germination of *Bacillus thuringiensis* var *kurstaki* in a Field Trial. Can. J. Microbiol. 48: 256-261 (2002).

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IIIA 7.1	Persistence and multiplication	
Reliability	1.	
Deficiencies	No.	

Table IIIA 7.1.1-01 The effect of *Bacillus thuringiensis* spores and crystals on soil organism counts

Organism	Counts per g of soil	
	Spores not added	Spores added
Total bacteria	5×10^7	1×10^8
Actinomycetes	2×10^5	1×10^6
Fungi	3×10^3	5×10^3
Nematodes	15	50
Protazoa	2	2

Table IIIA 7.1.1-02 Distribution and decline of viable *Bacillus thuringiensis* cells in two field soil irrigated naturally

Soil depth (cm)	Viable *Bacillus thuringiensis* cells per g of soil[1]							
	Plot A[2]					Plot B[3]		
Day	0	7	14	20	33	0	6	19
0 – 1	25.3×10^3	7.3×10^3	2.7×10^3	4.0×10^3	6.3×10^3	8.2×10^3	0.08×10^3	1.0×10^3
9 – 10	0	0	0	0	0	0	0	0
19 – 20	0	0	0	0	0	0	0	0

[1] Mean of duplicate experiments.
[2] Volcanic ash clay loam.
[3] Alluvium sand.

Table IIIA 7.1.1-03 Distribution and decline of viable *Bacillus thuringiensis* cells in two field soil irrigated artificially

Soil depth (cm)	Viable *Bacillus thuringiensis* cells per g of soil					
	Plot C[1]			Plot D[2]		
Day	0	7	15	0	7	15
0 – 0.5	1200×10^3	430×10^3	270×10^3	680×10^3	400×10^3	280×10^3
2.5 – 3	0	0	0.09×10^3	0	0.4×10^3	1×10^3
5.5 – 6	0	0	0	0	0	0.2×10^3
8.5 - 9	0	0	0	0	0	0

[1] Mean of duplicate experiments.
[2] Alluvium loam.
[3] Sand.

Table IIIA 7.1.1-04 Experimental conditions investigating the growth of *Bacillus thuringiensis* in soil

Factor	Level
pH	Unamended soil (pH 5.2) or amended (pH 7.3) with 30 mg $CaCO_3$/g (dry weight)
Moisture	0, -0.01, -0.1 or -1.0 MPa soil moisture capacity
Nutrient	Unsupplemented or supplemented with 5 mg yeast extract, 1 mg glucose and 0.25 mg K_2HPO_4/g (dry weight)
Microorganisms	Unsterilised or sterilized by autoclave (121°C for 20 minutes)

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by Rapporteur Member State
Date	September 2007
Materials and methods	Different methods have been used according to the biological investigated matter. They are currently found in the scientific literature.
Conclusion	In natural soils *Bti* exists predominantly in spore form that may persist for a long time (100-200 days). The spores do not germinate readily and germination is not influenced by soil pH, soil moisture, or by their pre-treatment with heat. *Bt* insecticidal crystal toxins degrade more rapidly, with a half-life of 9.5 to 8.5 days in grass and manure amended soils, respectively. The vegetative cells of *Bt* are not persistent in soil and decline rapidly under non-sterile conditions. The survival time may be as short as 1 to 2 days. Rapid degradation of vegetative cells and insecticidal toxins in soil, and poor germination of *Bti* spores in soil suggest *Bti* (Strain AM65-52) is fairly persistent and will not multiply in the soil environment *Bacillus thuringiensis* cells applied to field soils under natural conditions did not move appreciably through the soil profile. The very low mobility was attributed to adsorption onto clay minerals and silica. *Bacillus thuringiensis* parasporal crystal toxins are also rapidly bound to clay particles and will be similarly non-mobile in soil. *Bacillus thuringiensis* does not penetrate deeply into soil even after significant rainfalls, and therefore the potential for movement to groundwater must be considered minimal.
Reliability	2
Acceptability	Acceptable
Remarks	No direct studies on Strain AM65-52 have been performed..
	Comments from ...
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

SECTION IIIA 7	FATE AND BEHAVIOUR IN THE ENVIRONMENT	Official use only
IIIA 7.1	**Persistence and multiplication**	
IIIA 7.1.2 Water	*Bti* is not infectious and survives poorly in the environment resulting in a limited spread of the organism. Vegetative cells of *Bti* have a limited survival time in the environment and spores do not germinate readily, making it highly unlikely that *Bti* will multiply and colonise areas of intended use above levels that may occur naturally. *Bti* is not persistent in soil and water and airborne concentrations of the organism are expected to be negligible following application to water bodies. Further information is contained in a review by Glare and O'Callaghan and in the following study summaries.	
IIIA 7.1.2-01	**Water**	
Reference	Ohana, B., Marglit, J., Barak, Z. Fate of *Bacillus thuringiensis* subsp israelensis under simulated Field Conditions. Applied and Environmental Microbiology., Apr 1987. p 828-831.	
Data protection	No, published research	
Data owner	Not applicable	
Companies with letter of access	Not applicable	
Criteria for data protection	Not applicable	
Guideline study	Not applicable	
GLP	No	
Deviations	Not applicable	
Materials and Methods	The fate of *Bacillus thuringiensis* subsp. *israelensis* in aquatic systems in the presence or absence of soil was studied using laboratory simulated field water consisting of de-ionised water (45 L) that had been left to turn stagnant in the open air over a period of 8 weeks. A strain of *Bacillus thuringiensis* subsp. *israelensis* resistant to penicillin, streptomycin and tetracycline was developed in the laboratory to enable detection against background bacteria in water samples. *Bacillus thuringiensis* subsp. *israelensis* larvicidal activity was measured by tests carried out with third instar *Aedes aegypti* and by plating to determine the colony forming unit (cfu)/mL content. Experimental details are described in the report.	
Results	The larvicidal activity of *Bacillus thuringiensis* subsp. *israelensis* in stagnant water was reduced to almost 0% immediately on addition of soil to the test system. Viable bacteria also declined rapidly on addition of soil from a level of *ca* 1 x 10^5 cfu/mL initially to *ca* 1 x 10^3 cfu/mL after 15 minutes and to *ca* 1 x 10^2 cfu/mL from 45 minutes onwards. In contrast, in systems containing only water no reduction in larvicidal activity or viable bacteria was observed over the period of the test (6 hours). The influence of soil in the water samples was further investigated by analysing samples of suspended soil and water at 24 hour intervals over a 22 day period. The results showed that the number of viable bacteria remained constant at 5 x 10^4 cfu/L in the soil suspensions throughout this test, with the same samples showing no larvicidal activity. Filtration of the suspensions restored activity to *ca* 90% of the initial level; however viable bacteria counts were present at only *ca*	

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IIIA 7.1	**Persistence and multiplication**	
	8% of the initial level. A further test using stagnant water only was performed with *Bacillus thuringiensis* subsp. *israelensis* present at concentrations of 1 x 10^3 cfu/mL and 1.6 x 10^3 cfu/mL. Over a period of 30 days the number of viable counts remained constant, but after 3 to 4 days the larvicidal activity began to decline and continued such that between 0 and 20% of the initial activity remained after 11 days. A faster rate of decline and the complete loss of larvicidal activity were observed in samples containing the lower cfu/mL concentration.	
Applicant's Summary and conclusion	In water, contact of *Bacillus thuringiensis* subsp. *israelensis* with soil particles resulted in an immediate cessation of larvicidal activity but had no discernable effect on the number of viable bacteria. Disappearance of larvicidal activity was attributed to adsorption to soil particles with 99.8% of the bacteria found on soil particles after 45 minutes. Adsorption was weak with bacteria redetected after mechanical stirring. The bacterial count in the soil adsorbed fraction remained constant indicting that spores were viable but were not able to germinate and multiply in the soil. In contrast, in systems containing only water inhibition of the larvicidal activity was slow but was irreversible.	
Reliability	2.	
Deficiencies	No.	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by Rapporteur Member State
Date	September 2007
Materials and methods	Methods as currently found in scientific literature.
Conclusion	Vegetative cells of *Bti* have a limited survival time in the environment and spores do not germinate readily. The larvicidal activity of *Bti* in stagnant water was reduced to almost 0% after addition of soil to the test system. Viable bacteria also declined rapidly. In contrast, in systems containing only water no reduction in larvicidal activity or viable bacteria was observed over the period of the test (6 hours). Disappearance of larvicidal activity was attributed to adsorption to soil particles. In systems containing only water, inhibition of the larvicidal activity was slow but was irreversible.
Reliability	2
Acceptability	Acceptable
Remarks	No direct studies on Strain AM65-52 have been performed.
	Comments from ...
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

SECTION IIIA 7	**FATE AND BEHAVIOUR IN THE ENVIRONMENT**	**Official use only**
IIIA 7.1	**Persistence and multiplication**	
IIIA 7.1.2-02	**Water**	
Reference	Yousten, A. A., Genthner, F.J. Benfield, E.F. Fate of *Bacillus sphericus* and *Bacillus thuringiensis* serovar *israelensis* in the Aquatic Environment. Journal of the American Mosquito Control Association, Vol 8. No. 2. June 1992.	
Data protection	No, published research	
Data owner	Not applicable	
Companies with letter of access	Not applicable	
Criteria for data protection	Not applicable	
Guideline study	Not applicable	
GLP	No	
Deviations	Not applicable	
Materials and Methods	The adsorption of *Bacillus thuringiensis* subsp. *israelensis* to pond sediment, sand, clay silt and activated charcoal particles in aqueous media was studied using spores and toxins obtained from 'VectoBac' 12AS product. Experimental details are described in the report. The report also contains data relating to *Bacillus sphaericus*, however this information is not relevant and is not summarised.	
Results	The effect of adding particulate matter to aqueous solutions containing *Bacillus thuringiensis* subsp. *israelensis* is summarised in Table IIIA 7.1.2-01. All three particulate materials had settled during the test period. Sand had no effect on the settling of spores and toxins, whilst the clay silt and charcoal had a large effect, reducing concentrations from *ca* 10^5 spores/mL initially, to *ca* 10^3 and *ca* 10^2 spores/mL, respectively after 24 hours. In pond sediment, the spores of *Bacillus thuringiensis* subsp. *israelensis* settled slowly over a 24 hour period however insecticidal activity declined ten fold within 10 minutes and a further ten fold after 2 hours.	
Applicant's Summary and conclusion	*Bacillus thuringiensis* subsp. *israelensis* spores and toxins were sedimented effectively by clay silt and charcoal particles. Sand particles had little or no effect on the amount of spores and toxins in solution. Pond sediment significantly reduced the insecticidal activity of *Bacillus thuringiensis* subsp. *israelensis* although spore counts were largely unaffected.	
Reliability	2.	
Deficiencies	No.	

Table IIIA 7.1.2-01 Settling of *Bacillus thuringiensis* subsp. *israelensis* spores and toxins with particulate material

Particulate	Time (hours)	Dry weight (mg/ml)	Spores/mL
Sand	0	6.0	7.8×10^5
	24	0.03	9.6×10^5
Clay silt	0	6.0	4.8×10^5
	24	0.04	1.8×10^3
Charcoal	0	6.0	6.2×10^5
	24	0.01	9.5×10^2
Pond sediment	0	ND	4.5×10^6
	24	ND	3.8×10^6

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by Rapporteur Member State
Date	September 2007
Materials and methods	Methods as currently found in scientific literature.
Conclusion	*Bti* spores and toxins were sedimented effectively by clay silt and charcoal particles. Sand particles had little or no effect on the amount of spores and toxins in solution. Pond sediment significantly reduced the insecticidal activity of *Btis* although spore counts were largely unaffected.
Reliability	2
Acceptability	Acceptable
Remarks	No direct studies on Strain AM65-52 have been performed.
	Comments from ...
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

SECTION IIIA 7	FATE AND BEHAVIOUR IN THE ENVIRONMENT	Official use only
IIIA 7.1	**Persistence and multiplication**	
IIIA 7.1.3 Air	**JUSTIFICATION FOR NON-SUBMISSION OF DATA**	
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	*Bti* is not infectious and survives poorly in the environment resulting in a limited spread of the organism. Vegetative cells of *Bti* have a limited survival time in the environment and spores do not germinate readily, making it highly unlikely that *Bti* will multiply and colonise areas of intended use. *Bti* is not persistent in soil and water and airborne concentrations of the organism are expected to be negligible following application to contaminated water bodies and sewage.	
Undertaking of intended data submission [ ]	Not applicable.	
	EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	September 2007	
Evaluation of applicant's justification	*Bti* is not persistent in soil and water and airborne concentrations of the organism are expected to be negligible following application to contaminated water bodies and sewage.	
Conclusion	Acceptable	
Remarks	No studies on Strain AM65-52 have been performed. Studies on aerial applications have not been performed.	
	COMMENTS FROM OTHER MEMBER STATE	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

SECTION IIIA 7	FATE AND BEHAVIOUR IN THE ENVIRONMENT	Official use only
IIIA 7.2 Mobility	**JUSTIFICATION FOR NON-SUBMISSION OF DATA**	
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ] Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	*Bti* cells applied to field soils under natural conditions did not move appreciably through the soil profile. The lack of mobility was attributed to adsorption onto clay minerals and silica. *Bti* parasporal crystal toxins are also rapidly bound to clay particles and will be similarly non-mobile in soil. Further information regarding mobility of *Bti* in soil is presented under Point IIIA 7.1.1. *Bti* does not multiply in water bodies and the vegetative cells and insecticidal toxins are readily adsorbed onto particulate matter. Spread of the organism beyond the site of application is therefore considered unlikely.	
Undertaking of intended data submission [ ]	Not applicable.	
	EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	September 2007	
Evaluation of applicant's justification	Acceptable	
Conclusion	Due to adsorption of *Bti* cells and parasporal crystal toxins onto clay minerals and silica, their spread beyond the site of application can be considered unlikely.	
Remarks	No studies on Strain AM65-52 have been performed	
	COMMENTS FROM OTHER MEMBER STATE	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

SECTION IIIA 7	FATE AND BEHAVIOUR IN THE ENVIRONMENT	Official use only
IIIA 7.3 Summary and evaluation of fate and behaviour in the environment	*Bti* is not infectious and survives poorly in the environment resulting in a limited spread of the organism. Vegetative cells of *Bti* have a limited survival time in the environment and spores do not germinate readily, making it unlikely that *Bti* (Strain AM65-52) will multiply and colonise areas of intended use. *Bti* is fairly persistent or mobile in soil, is not persistent in water and airborne concentrations of the organism are expected to be negligible following application to contaminated water bodies and sewage.	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by Rapporteur Member State
Date	September 2007
Materials and methods	Not Applicable
Conclusion	*Bti* is fairly persistent or mobile in soil, it is not persistent in water and airborne concentrations of the organism are expected to be negligible following application to contaminated water bodies and sewage.
Reliability	2
Acceptability	Acceptable
Remarks	
	Comments from ...
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	