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

Dossier According to Directive 98/8/EC

Document IIIA

Study summaries – Active substance

SECTION IIIA 1	**IDENTITY OF THE MICRO-ORGANISM**		**Official use only**
IIIA 1.1 Applicant	**Name:**	Sumitomo Chemical Agro Europe SAS (for Valent BioSciences Corporation)	
	Address:	Parc d'affaires de Crecy, 2 rue Claude Chappe, FR – 69370 Saint-Didier-au-Mont-d'Or FRANCE	
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	e-mail:	[redacted]	
IIIA 1.2 Manufacturer			**Official use only**
IIIA 1.2.1 Producer of the Active Substance	**Name:**	Valent BioSciences Corporation	
	Address:	870 Technology Way Libertyville, Illinois 60048 USA	
	Contact person:	[redacted]	
	Telephone Number:	[redacted]	
	Fax Number:	[redacted]	
	e-mail	[redacted]	
IIIA 1.2.2 Manufacturer of the Active Substance	**Name:**	**Available in the confidential section**	
	Address:	**Available in the confidential section**	
	Contact person:	**Available in the confidential section**	
	Telephone Number:	**Available in the confidential section**	
	Fax Number:	**Available in the confidential section**	
	e-mail:	**Available in the confidential section**	

SECTION IIIA 1	**IDENTITY OF THE MICRO-ORGANISM**		**Official use only**
IIIA 1.3	**Name and species description, strain characterisation**		
IIIA 1.3.1 Common name of the micro-organism	*Bacillus* thuringiensis subsp. *israelensis* Serotype H-14 Strain AM65-52 (abbreviated to *Bti* (Strain AM65-52 in this dossier)		X
IIIA 1.3.2 Taxonomic name and strain and indication whether it is a stock varient, a mutant strain or a GMO.	**Species:**	*thuringiensis*	
	Subspecies:	*israelensis*	
	Serotype:	H-14	
	Strain:	AM65-52	
	Genus:	*Bacillus*	
	Family:	Bacillaceae	
	Bti (Strain AM65-52) originates from a natural wild strain of the bacteria and has not been genetically modified nor is it the result of a spontaneous or an induced mutation.		
IIIA 1.3.3	**ATCC safe deposit No.**	SD-1276 American Type Culture Collection.	

SECTION IIIA 1	**IDENTITY OF THE MICRO-ORGANISM**		**Official use only**
IIIA 1.3	**Name and species description, strain characterisation**		
Collection and culture reference number	**Strain designation**	*Bacillus thuringiensis* subsp *israelensis*, (Serotype H-14), Strain AM65-52, ATCC-1276	
	Date deposited at ATCC	1 Nov 1989	
	No. vials	12	
	Production strain/product	Production strain for 'VectoBac' WG	
	First description:	de Barjac (1978)[1]	

	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	None
Conclusion	Not applicable
Reliability	Not applicable
Acceptability	Not acceptable
Remarks	Method has been developed by the Applicant and shown to the RMS. The methodology should be made available at product authorization.
	Comments from ...
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

[1] Barjac, H. de. 1978. A new subspecies of *Bacillus thuringiensis* very toxic for mosquitoes: *Bacillus thuringiensis* var. *israelensis* serotype 14 (in French). C.R. Acad. SCi. (Paris) 286D: 797-800.

SECTION IIIA 1	IDENTITY OF THE MICRO-ORGANISM	Official use only
IIIA 1.3	**Name and species description, strain characterisation**	**X**
IIIA 1.3.4 Methods, procedures and criteria to establish presence and identity of the micro-organism	Characterisation of strain or serotype within *Bacillus thuringiensis* species is commonly performed using classical techniques such as; crystal morphology, biochemical reactions and bioassays. The technique of flagella serotyping with H-antigens has enabled researchers to classify *Bacillus thuringiensis* strains into at least 82 separate serovars. Serotype H-14 is classified as the serovar *israelensis*.	
IIIA 1.3.4-01	**Methods to establish presence and identity of the organism**	**Official use only**
Reference	Smith, R.A., Cooper, R.D. (1990). 'VectoBac' Technical Powder (EPA Registration Number 275-54) Product Chemistry Based on Bacillus thuringiensis, subspecies israelensis Strain AM65-52 (ATCC-SD-1276) as the Active Ingredient. Abbott Laboratories, unpublished report no. VTP-02. The information in this report is confidential to Valent BioSciences and is presented in the confidential attachment under Point IIIA 1.3.4-01.	

	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 20007
Materials and methods	Flagellar serotyping, biochemical testing, morphological testing antibiotic sensitivity pattern, toxin molecular weight.
Conclusion	Vectobac production culture Bti AM65-52 is characterised according to US EPA guidelines. Except for the deamination of phenylalanine, all biochemical test results were identical to the type *Bacillus thuringiensis* strain where results from the type strain were available. The flagella serotype was H-14. Bti (Strain AM65-52) was sensitive to some antibiotics but not others. Five extra-chromosomal bands with estimated molecular weights of less than 30 mDa and a single band greater than 30 mDa were observed in vegetative cells of Bti (Strain AM65-52). Crystalline protein inclusions isolated either from sporulated cultures or from technical grade active ingredient were composed primarily of the expected major proteins of molecular weight *ca* 135, 67 and 28 kDa.
Reliability	1
Acceptability	Acceptable: AM65-52 belongs to the declared genus, species, subspecies and serovar.
Remarks	(X) The Applicant has developed an adequate methodology which should be available at product authoarization.
	Comments from ...
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

SECTION IIIA 1	**IDENTITY OF THE MICRO-ORGANISM**	**Official use only**
IIIA 1.3	**Name and species description, strain characterisation**	
IIIA 1.3.4-02	**Methods to establish presence and identity of the organism**	
Reference	Lecadet, M.-M. *et al*. Updating the H-Antigen Classification of *Bacillus thuringiensis*. Journal of Applied Microbiology 1999, 86, 660-672.	
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 report contains a description of H-serotyping characterisation methods in addition to classical biochemical tests. Protein crystal inclusion morphology was determined by phase contrast microscopy and the protein profile was determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) analysis. Experimental details are described in the report.	
Results	The H-serotype technique has been found to be an efficient technology to classify strains of *Bacillus thuringiensis*. At the time of the report (1998) 69 serotypes, 13 sub-antigenic groups and 82 serovars among 3500 *Bacillus thuringiensis* isolates had been identified. The serovar *israelensis* is classified with the H-14 antigen. Biochemical classification is a useful tool used in conjunction with H-serotyping, with the biochemical characteristics grouped roughly into three major categories according to whether reactions are positive or negative for all serovars, or are discriminatory between serovars, as shown in Table IIIA 2.4-03. The morphology of crystal protein inclusions for the subspecies *israelensis* was spherical with sizes in the range 125-135-68-28 kDa.	
Applicant's Summary and conclusion	Flagella H-serotype identification techniques were reported to be an efficient technology to classify species of *Bacillus thuringiensis* along with classical biochemical tests. Crystal protein inclusions were identified by morphology and by SDS-PAGE protein analysis.	
Reliability	2.	
Deficiencies	No.	

Table IIIA 2.4-03 Biochemical characteristics of *Bacillus thuringiensis* serovars

Positive characters[1]	Negative characters[2]	Discriminant characters
Hydrolysis of: starch, gelatine, glycogen, esculin, N-acetyl-glucosamine	β-galactosidase Indole production Ornithine decarboxylase Lysine decarboxylase Tryptophan deaminase H_2S production	Arginine dihydrolase Urease Acetyl-methyl-carbinol production (VP) Nitrate reduction Utilisation of citrate
Fermentation of: glucose, fructose, maltose, trehalose, ribose	Fermentation of: galactose, lactose, mannitol	Fermentation of: sucrose, mannose, arbutin, salicin, cellobiose

[1] With the exception of H51 which was negative for starch, glycogen and ribose, and H67 which was negative for ribose.
[2] With the exception of H65 which was positive for galactose.

	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	H-serotyping characterization, crystal proteins morphology determined by phase contrast mocroscopy. Protein profile determined by SDS page.
Conclusion	Crystal protein inclusions were identified by morphology and by SDS-PAGE protein analysis
Reliability	2
Acceptability	Acceptable
Remarks	None
	Comments from ...
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

SECTION IIIA 1	**IDENTITY OF THE MICRO-ORGANISM**	**Official use only**
IIIA 1.3	**Name and species description, strain characterisation**	
IIIA 1.3.4-03	**Methods to establish presence and identity of the organism**	
Reference	Wie, S. *et al*. (1982). Enzyme-Linked Immunosorbent Assays for Detection and Quantitation of the Entomocidal Parasporal Crystalline Protein of *Bacillus thuringiensis* subsp. *kurataki and israelensis*. Applied and Environmental Microbiology, Volume 43, No. 4, April 1982, p.891 to 894.	
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	An enzyme-linked immunosorbent assay was used to detect and quantitate the parasporal crystal toxins of *Bacillus thuringiensis* subsp. *kurataki* and *israelensis*. The assay method described is extremely sensitive, accurate and highly specific. With this technique, crystalline insecticidal proteins from several subspecies of *B. thuringiensis* were compared. The dipteran crystal toxin produced by *Bacillus thuringiensis* subsp. *israelensis* was shown to share few epitopes with the lepidopteran toxin from *Bacillus thuringiensis* subsp. *kurstaki, tolworthi, berliner* and *alesti*. Two enzyme-linked immunosorbent assays (ELISAs) were used for the detection and quantitation of the entomocidal of the entomocidal prasporal crystalline proteins of *Bacillus thuringiensis* subsp. *kurstaki* and *israelensis*. Culture conditions and crystal preparation *Bacillus thuringiensis* subsp. *kurstaki, israelensis, tolworthi, berliner* and *alesti* were grown in a liquid medium containing glucose, yeast extract and salts. Insecticidal crystals were separated from spores and cellular debris on Renografin gradients. Crystal proteins were solubilised in alkali and dialysed overnight against 20 mM NaH_2PO_4 at pH 7.5. Preparation of antisera Antisera were obtained by inoculating New Zealand white albino rabbits with purified solubilised crystal toxin from *Bacillus thuringiensis* subsp. *kurstaki* and *israelensis*. To prepare anti-*B thuringiensis* subsp. *kurstaki* and *israelensis* crystal-alkaline phosphatase conjugates, antisera were precipitated with 40% saturated NH_4SO_4 and dialysed against 0.15 M NaCl - 0.1M NaH_2PO_4 (phosphate buffered saline) at pH 7.4. Alkaline phosphatase was then coupled to the immunoglobulins by the glutaraldehyde method. An indirect ELISA was developed to detect and quantitate crystal protein with commercially prepared anti-rabbit enzyme conjugate. The effect of antiserum concentration on colour intensity with various concentrations of the *Bacillus thuringiensis* subsp. *kurstaki* protein toxin which was adsorbed to the solid phase. Colour intensity (i.e. absorbance at 405 nm) was a function of crystal toxin concentration over a range of 0.03 to	

SECTION IIIA 1	IDENTITY OF THE MICRO-ORGANISM	Official use only
IIIA 1.3	**Name and species description, strain characterisation**	
IIIA 1.3.4-03	**Methods to establish presence and identity of the organism**	
	3 µg/mL). The lepidopterans toxins produced by *Bacillus thuringiensis* subsp. *tolworthi, berliner* and *alesti* also reacted with antiserum in a concentration dependent manner. However, the mosquito toxin of *Bacillus thuringiensis* subsp. *israelensis* did not react efficiently with antiserum prepared against the moth toxin of *Bacillus thuringiensis* subsp. *kurstaki.* Similarly, the negative control keyhole limpet hemocyanin (5 µg/mL) exhibited no reaction, even at antibody dilutions as low as 1:400.	
Results	It has previously been shown that the lepidopteran crystal toxins of *Bacillus thuringiensis* subsp. *kurstaki, tolworthi, berliner* and *alesti* are biochemically similar. Because no antigenic cross-reactivity was observed by indirect ELISA between the lepidopteran toxins and the dipteran toxin of *Bacillus thuringiensis* subsp. *israelensis,* the relationship of these proteins was further investigated. To determine how closely related these toxins are immunologically, two types of binding inhibition studies were performed. The ability to solubilise heterologous crystal toxins to inhibit the binding of anti-*B. thuringiensis* subsp. *kurstaki,* antibody to anti-*B. thuringiensis* subsp. *kurstaki,* crystal toxin adsorbed to the solid phase was studied. Crystal toxins from *B. thuringiensis kurstaki* and *berliner* were found to completely inhibit the binding reaction. Crystals from *B. thuringiensis* subsp. *tolworthi* and *alesti* also showed significant inhibition (91 and 79% respectively), but neither completely inhibited binding. *Bacillus thuringiensis* subsp. *israelensis* crystal toxin inhibited the binding less than 10% at concentrations as high as 1400 ng/mL. In the reciprocal experiment, it was found that none of the lepidopteran crystal toxins was an effective inhibitor (maximum inhibition ≅ 10%), including bovine serum albumin in the *Bacillus thuringiensis* subsp. *israelensis* system. To simplify the ELISA for quantification of crystal toxin, alkaline phosphotase was coupled directly to rabbit anti-*B. thuringiensis* subsp. *kurstaki* antibody, eliminating one antibody-antigen reaction step in the ELISA procedure. An enzyme-to-immunoglobulin ration of 1:2 was used in the preparation of this conjugate, and a concentration of 0.25 µg/mL of immunoglobulin was sufficient for a routine ELISA. The sensitivities of ELISA methods were similar and inhibition was linear over a wide range of concentrations (2 to 200 ng/mL).	
Applicant's Summary and conclusion	The results indicate that the crystal of *Bacillus thuringiensis* subsp. *israelensis* is biochemically different from the crystals of the four lepidopteran-toxic subspecies of *Bacillus thuringiensis.* Results of Ouchterlony double diffusion gel analyses and ELISA showed that crystal protein toxin from *Bacillus thuringiensis* subsp. *israelensis* also differs immunologically. Antiserum prepared against *Bacillus thuringiensis* subsp. *kurstaki* crystal protein reacted with solubilised crystals to yield an immunoprecipitate with protein from the four lepidopteran subspecies. Crystals from *Bacillus thuringiensis* subsp. *israelensis,* however, showed no cross-reactivity with anti-*B thuringiensis* subsp. *kurstaki* crystal antiserum, indicating that the toxin of *Bacillus thuringiensis* subsp. *israelensis* shares few epitopes with the toxins from the other four subspecies. The ELISA methods provide fast and efficient techniques for quantitation and differentiation of crystal toxins from *Bacillus thuringiensis* subsp. *israelensis and kurstaki.*	
Reliability	2.	

SECTION IIIA 1	IDENTITY OF THE MICRO-ORGANISM	Official use only
IIIA 1.3	Name and species description, strain characterisation	
IIIA 1.3.4-03	Methods to establish presence and identity of the organism	
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	Enzyme linked immunosorbent assay (ELISA)
Conclusion	The ELISA methods provide fast and efficient techniques for quantitation and differentiation of crystal toxins from *Bacillus thuringiensis* subsp. *israelensis and kurstaki*
Reliability	2
Acceptability	Acceptable
Remarks	Old but still effective method
	Comments from ...
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

SECTION IIIA 1	**IDENTITY OF THE MICRO-ORGANISM**	**Official use only**
IIIA 1.3	**Name and species description, strain characterisation**	
IIIA 1.3.4-04	**Methods to establish presence and identity of the organism**	**(X)**
Reference	Benson, T. (2005): Summary Report Genetic Comparison of *Bacillus thuringiensis* subsp. *israelensis* Strain AM65-52 to other Bacillus Strains using AFLP. Valent BioSciences, unpublished report number not stated. The information in this report is confidential to Valent BioSciences and is presented in the confidential attachment under Point IIIA 1.3.4-04.	

	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	Amplified fragment length polymorphism (AFLP) and methods reported in the published papers by Hill *et al*. (2004), Ticknor *et al*. (2001) and Jackson *et al* (1999)
Conclusion	DNA analysis shows that Bti (Strain AM65-52) is in a phylogenetic grouping containing many *Bacillus thuringiensis* strains which are not toxigenic to vertebrates. It is distinctly separate from the cluster of pathogenic and toxigenic *Bacillus* isolates
Reliability	1/2
Acceptability	Acceptable
Remarks	(X) The method does not provide unequivocal evidence for identity of the strain with respect to other strain below 2% genetic distance. The method shows its potential for discrimination among strains, but does not provide the actual identity of AM65-52. However a method for unequivocal strain identification has now been developed, which should be made available at product authorization.
	Comments from ...
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

SECTION IIIA 1	**IDENTITY OF THE MICRO-ORGANISM**	**Official use only**
IIIA 1.4	**Specification of material used for manufacturing formulated products**	
IIIA 1.4.1 Content of the micro-organism	The technical grade of *Bti* (Strain AM65-52) is a fermentation slurry that contains the bacillus, spores and insecticidal toxins and solid residues from the fermentation. Fermentation residues will include the original components of the fermentation medium, plus metabolic and excretion products from the growing bacteria. The fermentation slurry contains nominally 14% *Bti* (Strain AM65-52), with high and low limits of 20% and 8%, respectively. Further information regarding the composition of the micro-organism is confidential to Valent BioSciences and is presented in the confidential attachment under Point IIIA 1.4.1.	

	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	Not applicable
Reliability	1
Acceptability	acceptable
Remarks	none
	Comments from ...
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

SECTION IIIA 1	**IDENTITY OF THE MICRO-ORGANISM**	**Official use only**
IIIA 1.4	**Specification of material used for manufacturing formulated products**	
IIIA 1.4.2	**Identity and content of impurities, additives and contaminating micro-organism**	**(X)**
IIIA 1.4.2.1 Impurities	Human or mammalian pathogen impurities of *Bti* (Strain AM65-52) may occur as beta-exotoxins, bacterial contaminants or emetic/diarrhoeal enterotoxins. Beta-exotoxins are adenosine triphosphate (ATP) analogues that are water soluble and heat stable metabolites formed during the vegetative growth phase of some *Bacillus thuringiensis* strains. They are inhibitors of RNA polymerase and act competitively with natural ATP in various biological processes and as such can be toxic. Enterotoxins are considered important because they are characteristic of the *Bacillus cereus* species which is closely related to *Bacillus thuringiensis*. Information concerning impurities is confidential to Valent BioSciences and is presented in the confidential attachment under Point IIIA 1.4.2.1.	

	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	Microbial testing performed according to internal laboratory procedure. Absence of beta-exotoxins monitored by fly larvae and mouse safety test. Culture fermentation process checked by standard microscopic and agar plating methods.
Conclusion	Conclusion considered confidential by the Applicant
Reliability	1
Acceptability	Acceptable
Remarks	(X) The Methods are twenty years old, and there are better, quicker, and more reliable DNA methods for identification of impurities. Agar plating methods do not provide evidence for the presence of non-culturable contaminants, or contaminants difficult to be cultured
	Comments from ...
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

SECTION IIIA 1	IDENTITY OF THE MICRO-ORGANISM	Official use only
IIIA 1.4	**Specification of material used for manufacturing formulated products**	
IIIA 1.4.2	**Identity and content of impurities, additives and contaminating micro-organism**	(X)
IIIA 1.4.2.2 Additives	Information concerning additives in *Bti* (Strain AM65-52) is confidential to Valent BioSciences and is presented in the confidential attachment under Point IIIA 1.4.2.2.	

	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	Not applicable
Reliability	Not applicable
Acceptability	Not applicable
Remarks	(X) Specifications considered confidential, but point IIIA 1.4.2.2 does not contain details
	Comments from ...
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

SECTION IIIA 1	**IDENTITY OF THE MICRO-ORGANISM**	**Official use only**
IIIA 1.4	**Specification of material used for manufacturing formulated products**	
IIIA 1.4.2	**Identity and content of impurities, additives and contaminating micro-organism**	
IIIA 1.4.2.3 Contaminating micro-organisms	'VectoBac' products are manufactured by submerged pure culture fermentation of the organism *Bti* (Strain AM65-52). The manufactured technical slurry of *Bti* (Strain AM65-52) is considered to be free of pathogenic microbial impurities according to the data presented under Point IIIA 1.4.2.1 and Point IIIA 1.4.3.	

	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	Microbial testing performed according to internal laboratory procedure. Absence of beta-esotoxins monitored by fly larvae and mouse safety test. Culture fermentation process checked by standard microscopic and agar plating methods. Potency determined by bioassay
Conclusion	The manufactured technical grade active ingredient Bti (Strain AM65-52) is considered to be free of hazardous amounts of human and/or animal pathogens.
Reliability	1
Acceptability	Acceptable
Remarks	None
	Comments from ...
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

SECTION IIIA 1	IDENTITY OF THE MICRO-ORGANISM	Official use only
IIIA 1.4	**Specification of material used for manufacturing formulated products**	
IIIA 1.4.3	**Analytical profile of batches**	(X)
References	Batch data are contained in the following reports. IIIA1.4.3-01 - Benzon, G.L. (2002) Analysis of Dipteran Biopotency of 'VectoBac' HP TP (ABG-6164F). Benzon Research, unpublished report no. VB0102P. IIIA 1.4.3-02 - Isaacson, J.A. (1991) Analysis of Beta-exotoxin (thuringiensin) Content of Five Lots of 'VectoBac' TP by Housefly Bioassay. Abbott Laboratories, unpublished report no. 910-9011. IIIA 1.4.3-03 - Coddens, M. (1990) 'VectoBac' Technical Powder (EPA Registration Number 275-54) Product chemistry Based on *Bacillus thuringiensis*, subspecies *israelensis* , Strain AM65-52 (ATCC-SD-12796) as the Active Ingredient. Abbott Laboratories, unpublished report no. VTP-03. IIIA 1.4.3-04 - Smith, R.A., Cooper, R.D. (1990). 'VectoBac' Technical Powder (EPA Registration Number 275-54) Product Chemistry Based on *Bacillus thuringiensis*, subspecies *israelensis* Strain AM65-52 (ATCC-SD-1276) as the Active Ingredient. Abbott Laboratories, unpublished report no. VTP-02. IIIA 1.4.3-05 – Brand, R. (1998) Bioburden analysis of 'VectoBac' WDG (ABG-6490). Abbott Laboratories, unpublished report no. 054-97. The information contained in these reports is confidential to Valent BioSciences and is presented in the confidential attachment under Point IIIA 1.4.3.	

	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 considered Confidential by the Applicant
Conclusion	Conclusions considered confidential by the Applicant. They are internal to the Applicant's Laboratories
Reliability	1
Acceptability	(X) Although acceptable, all the methods are rather old
Remarks	(X) The data, not only the methods, are rather old. It would be desirable to have these tests repeated at given, regular intervals (3 or more years for example).
	Comments from ...
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	