| **SECTION IIIA 9** | **CLASSIFICATION AND LABELLING** | **Official use only** |
|---|---|---|
| The summary of findings from laboratory studies, published literature and regulatory reviews, in conjunction with the medical surveillance reports from production areas is that *Bacillus thuringiensis* subsp. *israelensis*, Serotype H-14, Strain AM65-52 poses no quantifiable risk to human health in respect of its use as a microbial insecticide.<br><br>*Bacillus thuringiensis* subsp. *israelensis*, Serotype H-14, Strain AM65-52 is therefore unlikely to cause human disease and can be classified as a Group 1 biological agent according to Article 2 of Directive 2000/54/EC. The use of the biohazard symbol on products containing *Bacillus thuringiensis* subsp. *israelensis*, Serotype H-14, Strain AM65-52 is not required. | | |

| | **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** | Acceptable |
| **Remarks** | None |
| | **Comments from ...** |
| **Date** | |
| **Results and discussion** | |
| **Conclusion** | |
| **Reliability** | |
| **Acceptability** | |
| **Remarks** | |

| **SECTION IIIA 10** | **SUMMARY AND EVALUATION OF SECTIONS 1 TO 9** | **Official use only** |
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| The summary and evaluation of Sections 1 to 9 is presented in the IIA summary for the active micro-organism. | | |

| | **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** | The summary for the active microorganism is presented in the document IIA (Effect and exposure assessments, Active Substance) which consists of 16 pages.<br><br>*Bti* AM65-52 is a Gram positive, spore forming rod-shaped bacterium that produces a crystalline protein inclusion which is toxic to larvae of some *Dipteran* insects upon ingestion. The mode of action of *Bti* AM65-52 results from toxic proteins contained in the crystalline protein inclusion. The crystals are taken up by the target insect larvae via ingestion and under the alkali conditions present in the larvae gut the crystal dissolves releasing the active protein δ-endotoxins (Cry4Aa1, Cry4Ba1, Cry10Aa1, Cry11Aa1 and Cyt1Aa1) that induce disintegration of the larvae gut epithelium and consequent death of the larvae.<br><br>As for the efficacy, the available information from field experiments shows that 'VectoBac' WG is highly effective under a range of conditions against a variety of mosquito species including; *Aedes albopictus, Culex pipiens, Ochlerotatus caspius, Aedes vigilax, Aedes vexans, Aedes caspius, Culiseta annulata, Aedes sticticus* and *Aedes rossicus/cinereus*. The tests were performed at rates up to 500 g/ha, with the target species present at between the 1st and early 4th instar larval growth stage. Typically greater than 95% control was observed after 48 hours. Information from field experiments to show that *Bti* AM65-52 is highly effective against black flies (*Simuliidae*) and a range of filter flies including; *Sylvicola spp*, *Metriocnemus hygropetricus*, *Orthocladius fuscinanus*, *Psychoda alternata* and *P. severini*. In conclusion, 'VectoBac' WG (*Bti* AM65-52) is sufficiently effective against mosquito, black flies and filter flies and the results of the efficacy studies support the label recommendations.<br><br>'VectoBac' WG (*Bti* AM65-52) is not an adulticide and application when larvae are present up to the early 4th instar growth stage is necessary for effective control.<br><br>As for toxicity related to metabolites of *Bti* – or, more specifically, to the four protein complex involved in parasporal body toxicity, published literature indicates the highly species specific nature of the *Bti* δ-endotoxin, the lack of toxic effects in warm-blooded organisms and the lack of activation in the non-alkaline gut environments of mammals.<br><br>In a range of toxicological studies, completed using *Bti*, experimental infection of mice, rats, guinea pigs and rabbits was attempted by various routes. Single and repeat administration tests revealed an absence of acute or prolonged toxicity at doses of approximately $10^7$ to $10^8$ bacteria per animal. There were no indications of anaphylaxis in guinea pigs and repeated passage through mice induced no virulent response. Repeat administration of a dose in the order of $10^{11}$ or $10^{12}$ bacteria per rat/mouse for three weeks resulted in no pathogenicity. In none of these tests was there evidence of pathological symptoms, disease or mortality. Behaviour and weight gain were unaffected by treatment and necropsy revealed no macroscopic effects. The re-isolation tests for various organs were negative. It was conclude that *Bti* was well tolerated by the test species used, showed no propensity to multiply within the host and was rapidly eliminated without causing adverse effects.<br><br>An assessment of the health effects of *Bti* on operators involved in the fermentation |

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| | process and other persons likely to be exposed to the material is presented as a summary of medical surveillance. The Medical Director responsible for the plant confirmed no abnormalities and no human health related or other adverse reactions to *Bti*.<br><br>As for human infection in relation to *Bacillus* species the overall results indicate that *B. thuringiensis* may be responsible for opportunistic infections and that the possibility of a human infection with *Bacillus thuringiensis* is limited only to severe immunocompromised patients. There are no indications that *Bti* AM65-52 is involved in human pathogenicity, infectivity or toxicity.<br><br>The overall assessment of the acute toxicity/infectivity pathogenicity studies on *Bti* (AM65-52) indicates no evidence of toxicity/infectivity or pathogenicity for the human health.<br><br>*Bti* AM65-52 technical powder should be considered as a potential human sensitizer, at concentration above 5,0% w/v, as clearly demonstrate in a experimental test study on guinea pigs, according to the Buehler protocol. The formulated material, at 37.5% did not present sensitization potential in a Maximization study.<br><br>Rapid degradation of *Bti* AM65-52 vegetative cells and insecticidal toxins in soil and poor germination of *Bti* AM65-52 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.<br><br>In water, contact of *Bti* with soil particles resulted in an immediate cessation of larvicidal activity but has no discernable effect on the number of viable bacteria. Disappearance of larvicidal activity is attributed to adsorption of the insecticidal toxins and vegetative cells to soil particles with rapid and virtually complete adsorption of the bacteria onto soil particles. Adsorption was however reversible with mechanical stirring. Soil adsorbed spores remain viable but do not readily germinate and multiply. In systems containing only water, inhibition of larvicidal activity was slow but was irreversible showing a gradual degradation of the insecticidal toxins.<br><br>*Bti* AM65-52 is not infectious and survives poorly in the environment resulting in a limited spread of the organism. Vegetative cells and insecticidal toxins of *Bti* have a limited survival time in the environment and *Bti* AM65-52 spores do not germinate readily, making it highly unlikely that *Bti* AM65-52 will multiply and colonise areas of intended use above levels that may occur naturally. *Bti* vegetative cell and insecticidal toxins are not persistent or mobile in soil, are not persistent in water and airborne concentrations of are expected to be negligible following application to water bodies and sewage. |
| **Reliability** | Not applicable |
| **Acceptability** | acceptable |
| **Remarks** | none |
| | **Comments from ...** |
| **Date** | |
| **Results and discussion** | |
| **Conclusion** | |
| **Reliability** | |
| **Acceptability** | |
| **Remarks** | |