Bacillus thuringiensis israelensis (Bti) in drinking-water

Background document for development of
WHO *Guidelines for Drinking-water Quality*

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This document is based on Environmental Health Criteria (EHC) 217 (IPCS, 1999) and other background information. It should be noted that EHC 217 is on Bacillus thuringiensis, whereas the primary focus of this background document is on the subspecies Bacillus thuringiensis israelensis (Bti). Two Bti (strain AM65-52) products (WG, water-dispersible granule; and DT, ready-to-use tablet) have been evaluated by the WHO Pesticide Evaluation Scheme (WHOPES) and recommended as mosquito larvicides, including their use against container-breeding mosquitoes (WHO, 2004, 2006). Quality control specifications and efficacy evaluations for Bti WG have been published (WHO, 2007). WHO recommendations on the use of pesticides in public health are valid only if linked to WHO specifications for their quality control.

1. GENERAL DESCRIPTION

1.1 Identity

Bacillus thuringensis (Bt) is a facultative anaerobic, Gram-positive bacterium that forms characteristic protein inclusions adjacent to the endospore. Bt is genetically indistinguishable from *Bacillus cereus* (Bc). However, Bt has the ability to produce parasporal crystalline inclusions, which are toxic for certain invertebrates, especially species of insect larvae belonging to the insect orders Coleoptera, Diptera and Lepidoptera. The parasporal inclusions are formed by different insecticidal crystal proteins (ICP). The crystals have various shapes (bipyramidal, cuboidal, flat rhomboid, spherical or composite with two crystal types), depending on their ICP composition.

The basic phenotypic taxon of Bt is the subspecies, identified by its flagellar antigen (H) or serotype. By 1998, 67 subspecies had been described. The subspecies *israelensis* (Bti) is the focus of this background document. Its properties, activities and applications have been reviewed elsewhere (Lacey, 2007).

The existence of parasporal inclusions in Bt was first noted in 1915, but their protein composition was not delineated until the 1950s. A property of most of the parasporal inclusions is the crystalline fine structure. However, Bt subspecies can synthesize more than one inclusion, which may contain different ICPs. A partial correlation between crystal morphology, ICP composition and bioactivity against target insects has been established. ICPs have also been called delta endotoxins. However, because the term endotoxin usually refers to toxins associated with the outer membranes of Gram-negative bacteria, comprising a core lipopolysaccharide, lipid A and somatic (O) antigens, this term is not used here.

During vegetative growth, various Bt strains produce an assortment of antibiotics, enzymes, metabolites and toxins, including Bc toxins, that may have detrimental effects on both target organisms and non-target organisms. Beta-exotoxin, a heat-stable nucleotide, is produced by some Bt subspecies during vegetative growth and may contaminate the products. Beta-exotoxin is toxic for almost all forms of life, including humans and the target insect orders.

1.2 Insecticidal preparations

It is essential that Bti for larvicidal use be prepared under carefully controlled conditions and properly assayed before use for evidence of potency, for excessive levels of expressed Bti constituents or metabolites that are toxic and for contamination by other undesirable microbes. These specifications for quality control are available in WHO (2007).

Bt spore counts do not accurately reflect the insecticidal activity of a Bt strain or Bt product. The potency (international toxic units [ITU]/mg) of each Bt product is bioassayed using an international standard that uses a specific test insect. It is also tested for the presence of Bt ICP (based on a electrophoretic analysis of molecular weight) and plasmid profile (based on electrophoretic analysis).

However, the tests for Bti potency do not identify other undesirable constituents or metabolites or other microbial contaminants. Current WHO specifications and evaluations are for only a limited number of impurities, specifically water, microscopic evidence of material or microbes other than the target bacteria, and culture-based testing for four other bacteria or bacteria groups. *Staphylococcus aureus*, *Salmonella* species, *Pseudomonas aeruginosa* and *Escherichia coli* are tested for in product quantities of only 0.1 or 0.2 g. There is no testing for other microbes, toxins or other chemicals as contaminants. However, the presence of contaminating faecal bacteria, such as *Streptococcus faecalis* and *S. faeceum*, has been previously reported in some Bt products (Kane & Eaton, 1987; Swadener, 1994).

1.3 Major uses

Preparations of Bti are widely used against a number of insect species, but specific activity against disease vector species such as mosquitoes and blackflies has resulted in its use in water. It is approved under WHOPES for use in vector control and can be used in drinking-water that will receive little or no further treatment.

1.4 Mode of action

The sporulated Bt with ICP or spore–ICP complexes must be ingested by a susceptible insect larva. The efficacy of the ICP depends on the solubilization in the midgut, the conversion of the protoxin to the biologically active toxin by proteolytic enzymes, specific membrane receptor binding by the C-terminal domain of the active toxin, and pore formation by the *N*-terminal domain with subsequent lysis of the epithelial cells. Spore germination and proliferation of the vegetative cells into the haemocoel may result in a septicaemia, contributing to the cause of death. Receptor binding by the ICP is the major determinant of host specificity by the different Bt ICPs.

1.5 Environmental fate

Bti is often applied directly to water for the control of mosquitoes and blackflies. Rapid sedimentation in all but the fastest flowing streams is regarded as an important limitation on the efficacy of such applications. However, special Bti formulations

have been developed to prolong the residence time of Bti at the surface or in the water column, where target insects feed.

In particular, it has been demonstrated that the sedimentation of Bti is facilitated by adsorption onto particulate material. Bti has persisted as long as 5 months in cold water, and adsorption to particulate matter in water facilitates persistence (Boisvert & Boisvert, 1999). It has been found that spores may persist for at least 22 days in sediments, and the spores may be mobilized with such sediments during floods. However, Bti has been less effective in habitats with high algal content and in fast flowing streams, primarily because of the inability to penetrate algal mats and dilution effects (Shililu et al., 2003). Because spores of Bti have remained viable for shorter periods when suspended in moving water than when in static bottles, static laboratory trials may overestimate the longevity of these spores in the environment (Yousten et al., 1992).

Carcasses of mosquito larvae killed by Bti have been shown to allow for the complete growth cycle (germination, vegetative growth and sporulation), thus becoming toxic themselves to scavenging yellow fever mosquito (*Aedes aegypti*) larvae. Contact of Bti with mud can result in an immediate disappearance of larvicidal activity, but it has little influence on spore viability. The cessation of toxicity was found to be caused by bacterial adsorption to soil particles, but the inactivation could be reversed by washing the mud away.

2. EXPOSURE IN DRINKING-WATER

While it is probable that humans are exposed to trace levels of the toxin from its natural occurrence in the environment, the major source will be drinking-water when Bti is used for vector control in drinking-water containers or reservoirs.

Bti has not been isolated from any drinking-water supplies, but it is not clear whether investigations of Bti applied specifically to drinking-water have been conducted.

3. TOXICOLOGICAL SUMMARY

Studies on mammals, particularly those on laboratory animals, have evaluated possible infectivity and toxicity of various Bt preparations, which include the ICPs, vegetative cells and spores. The ICPs, spores and vegetative cells of the Bt subspecies, which were administered by different routes, were mostly non-pathogenic and non-toxic to the various animal species tested. The vegetative cells and/or spores of Bt were demonstrated to persist for weeks without causing adverse effects. Bt has not been observed to adversely affect birds, fish or many other non-target aquatic vertebrates tested in a large number of laboratory and field studies.

More detailed information on the toxicology of Bt may be found in IPCS (1999).

4. PRACTICAL ASPECTS

4.1 Analytical methods and analytical achievability

4.1.1 International bioassay for ICPs

The final formulation of each Bti product is bioassayed against an accepted international standard using a specific test insect. Its potency is defined in ITU/mg product. The standardization allows comparison of different formulations in the laboratory. Currently, the larvicidal activity is expressed in terms of lethal doses (LD_{50}) or lethal concentrations (e.g. LC_{50}, LC_{90}) according to the bioassay method used. For example, when susceptible mosquito larvae are exposed to Bti ICP, they have an LC_{50} of approximately 10 ng/ml water. A Bti whole culture (unpurified and undiluted culture) gives an LC_{50} of approximately 10^3 cells/ml for susceptible mosquito larvae, whereas a concentrated 10^9 cells/ml culture does not affect any laboratory mammals exposed by various routes.

4.1.2 Manufacturing practices, quality control and quality assurance

There is no evidence to document that Bti production is done under Good Manufacturing Practices (GMP) or employs the widely accepted principles of hazard analysis and critical control point (HACCP) conditions, as would be required for pharmaceuticals, foods, food supplements or additives to drinking-water. Therefore, the bacteriological quality of Bti is questionable based on the limited requirements of testing for other bacteria. There are no quality requirements for Bti, based on testing for bacterial impurities, such as faecal streptococci, enterococci, most Gram-negative enteric bacterial pathogens and faecal indicator bacteria, and other potentially pathogenic or otherwise undesirable bacteria. Therefore, it is questionable as to whether Bti is produced under suitable conditions to ensure its purity and safety as a drinking-water additive and is of sufficient quality with respect to contaminants or impurities to exclude such microbial contaminants, as would be required for other additives to drinking-water.

4.2 Use for vector control in drinking-water sources

Preparations of Bti are widely used against a number of insect species, but specific activity against disease vector species such as mosquitoes and blackflies has resulted in its use in water. It is approved under WHOPES for use in vector control and can be used in drinking-water that will receive little or no further treatment. It is essential that Bti for larvicidal use be prepared under carefully controlled conditions and properly assayed before use for evidence of potency, for excessive levels of expressed Bti constituents or metabolites that are toxic and for contamination by other undesirable microbes or microbial constituents and products, such as other microbial toxins besides those of Bti.

5. GUIDELINE VALUE

Bti itself is not considered to pose a hazard to humans through drinking-water. Therefore, it is not considered necessary or appropriate to establish a guideline for it. However, the absence of contaminating bacteria or other impurities cannot be assured

under currently allowed production practices and post-production testing requirements as quality assurance and quality control measures. Therefore, stricter and more comprehensive requirements for production and post-production testing and better defined criteria for purity and safety are recommended to minimize human health risks from possible drinking-water exposures.

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