

Bacillus thuringiensis israelensis (Bti) in drinking-water

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

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Preface

One of the primary goals of the World Health Organization (WHO) and its Member States is that “all people, whatever their stage of development and their social and economic conditions, have the right to have access to an adequate supply of safe drinking water.” A major WHO function to achieve such goals is the responsibility “to propose ... regulations, and to make recommendations with respect to international health matters”

The first WHO document dealing specifically with public drinking-water quality was published in 1958 as *International Standards for Drinking-water*. It was subsequently revised in 1963 and in 1971 under the same title. In 1984–1985, the first edition of the WHO *Guidelines for Drinking-water Quality* (GDWQ) was published in three volumes: Volume 1, Recommendations; Volume 2, Health criteria and other supporting information; and Volume 3, Surveillance and control of community supplies. Second editions of these volumes were published in 1993, 1996 and 1997, respectively. Addenda to Volumes 1 and 2 of the second edition were published in 1998, addressing selected chemicals. An addendum on microbiological aspects reviewing selected microorganisms was published in 2002. The third edition of the GDWQ was published in 2004, the first addendum to the third edition was published in 2006 and the second addendum to the third edition was published in 2008. The fourth edition will be published in 2011.

The GDWQ are subject to a rolling revision process. Through this process, microbial, chemical and radiological aspects of drinking-water are subject to periodic review, and documentation related to aspects of protection and control of public drinking-water quality is accordingly prepared and updated.

Since the first edition of the GDWQ, WHO has published information on health criteria and other supporting information to the GDWQ, describing the approaches used in deriving guideline values and presenting critical reviews and evaluations of the effects on human health of the substances or contaminants of potential health concern in drinking-water. In the first and second editions, these constituted Volume 2 of the GDWQ. Since publication of the third edition, they comprise a series of free-standing monographs, including this one.

For each chemical contaminant or substance considered, a lead institution prepared a background document evaluating the risks for human health from exposure to the particular chemical in drinking-water. Institutions from Canada, Japan, the United Kingdom and the United States of America (USA) prepared the documents for the fourth edition.

Under the oversight of a group of coordinators, each of whom was responsible for a group of chemicals considered in the GDWQ, the draft health criteria documents were submitted to a number of scientific institutions and selected experts for peer review. Comments were taken into consideration by the coordinators and authors. The draft documents were also released to the public domain for comment and submitted for final evaluation by expert meetings.

During the preparation of background documents and at expert meetings, careful consideration was given to information available in previous risk assessments carried out by the International Programme on Chemical Safety, in its Environmental Health Criteria monographs and Concise International Chemical Assessment Documents, the International Agency for Research on Cancer, the Joint FAO/WHO Meetings on Pesticide Residues and the Joint FAO/WHO Expert Committee on Food Additives (which evaluates contaminants such as lead, cadmium, nitrate and nitrite, in addition to food additives).

Further up-to-date information on the GDWQ and the process of their development is available on the WHO Internet site and in the current edition of the GDWQ.

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The work of the following working group coordinators was crucial in the development of this document and others contributing to the fourth edition:

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The draft text was discussed at the Expert Consultation for the fourth edition of the GDWQ, held on 19–23 June 2008. The final version of the document takes into consideration comments from both peer reviewers and the public. The input of those who provided comments and of participants at the meeting is gratefully acknowledged.

The WHO coordinators were Mr R. Bos and Mr B. Gordon, WHO Headquarters. Ms C. Vickers provided a liaison with the International Programme on Chemical Safety, WHO Headquarters. Mr M. Zaim, WHO Pesticide Evaluation Scheme, Vector Ecology and Management, WHO Headquarters, provided input on pesticides added to drinking-water for public health purposes.

Ms P. Ward provided invaluable administrative support at the Expert Consultation and throughout the review and publication process. Ms M. Sheffer of Ottawa, Canada, was responsible for the scientific editing of the document.

Many individuals from various countries contributed to the development of the GDWQ. The efforts of all who contributed to the preparation of this document and in particular those who provided peer or public domain review comments are greatly appreciated.

Acronyms and abbreviations used in the text

Bc	<i>Bacillus cereus</i>
Bt	<i>Bacillus thuringiensis</i>
Bti	<i>Bacillus thuringiensis israelensis</i>
EHC	Environmental Health Criteria
FAO	Food and Agriculture Organization of the United Nations
GDWQ	<i>Guidelines for Drinking-water Quality</i>
GMP	Good Manufacturing Practices
HACCP	hazard analysis and critical control point
ICP	insecticidal crystal proteins
ITU	international toxic units
LC ₅₀	median lethal concentration
LC ₉₀	lethal concentration for 90% of test organisms
LD ₅₀	median lethal dose
USA	United States of America
WHO	World Health Organization
WHOPES	WHO Pesticide Evaluation Scheme

Table of contents

1. GENERAL DESCRIPTION	1
1.1 Identity	1
1.2 Insecticidal preparations	2
1.3 Major uses	2
1.4 Mode of action	2
1.5 Environmental fate	3
2. EXPOSURE IN DRINKING-WATER	3
3. TOXICOLOGICAL SUMMARY	3
4. PRACTICAL ASPECTS	4
4.1 Analytical methods and analytical achievability	4
4.1.1 International bioassay for ICPs	4
4.1.2 Manufacturing practices, quality control and quality assurance	4
4.2 Use for vector control in drinking-water sources	4
5. CONCLUSIONS	5
6. REFERENCES	5

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 World Health Organization (WHO) Pesticide Evaluation Scheme (WHOPES) and recommended as mosquito larvicides, including their use against container-breeding mosquitoes (WHO, 2004, 2006). WHO specifications for quality control of 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.

WHO specifications for formulations, unless otherwise stated, encompass the products of all formulators legitimately able to certify that their products contain only active ingredient sourced from a manufacturer to whom the WHO specification for technical material/technical concentrate applies. Buyers and/or regulatory authorities should demand such certification and ensure both that it is valid and that the products fully comply with the physical and chemical requirements of the WHO specifications. The safety of the formulants used in making the final product should be considered by national authorities for products intended for use in potable water.

1. GENERAL DESCRIPTION

1.1 Identity

Bacillus thuringiensis (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 cannot identify all possible 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. faecium*, has been previously reported in some Bt products (Kane & Eaton, 1987; Swadener, 1994).

1.3 Major uses

Preparations of Bti are widely used against mosquitoes, chironomids and blackflies, and this specific activity against disease vector species has resulted in the use of Bti in water. Bti is recommended under WHOPES for use in vector control, including against container-breeding mosquitoes (WHO, 2004, 2006), and can be used in drinking-water that will receive little or no further treatment for control of *Aedes aegypti* (IPCS, 1999).

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, Genthner & Benfield, 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 persistence 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 (LD₅₀) or lethal concentrations (e.g. LC₅₀, LC₉₀) according to the bioassay method used. For example, when susceptible mosquito larvae are exposed to Bti ICP, they have an LC₅₀ of approximately 10 ng/ml water. A Bti whole culture (unpurified and undiluted culture) gives an LC₅₀ of approximately 10³ cells/ml for susceptible mosquito larvae, whereas a concentrated 10⁹ 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, it is possible for the bacteriological quality of Bti to be questionable, based on the current testing requirements for other bacteria. There are only limited and, therefore, not comprehensive quality requirements for Bti, based on testing for bacterial impurities. Important health and sanitation-related bacteria, such as faecal streptococci, enterococci, most Gram-negative enteric bacterial pathogens and faecal indicator bacteria, as well as other potentially pathogenic or otherwise undesirable bacteria are not tested for. Therefore, it is vital that 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 mosquitoes, chironomids and blackflies, and this specific activity against disease vector species has resulted in the use of Bti in water. Bti is recommended 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.

WHO specifications for formulations, unless otherwise stated, encompass the products of all formulators legitimately able to certify that their products contain only

active ingredient sourced from a manufacturer to whom the WHO specification for technical material/technical concentrate applies. Buyers and/or regulatory authorities should demand such certification and ensure both that it is valid and that the products fully comply with the physical and chemical requirements of the WHO specifications. This is particularly important for any pesticide formulations to be used in drinking-water in containers in view of the direct exposure of humans.

5. CONCLUSIONS

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 its use for controlling vector larvae in drinking-water. However, it is vital that authorities can be assured that Bti has been prepared to the highest quality and hygienic standards under appropriate conditions that will meet the WHOPES specifications. It is important that the possible risks are set against the risks from vector-borne diseases such as dengue fever.

Application should be carried out by trained applicators and Bti used in conjunction with other approaches to vector control, including exclusion of mosquitoes from containers and other control options.

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