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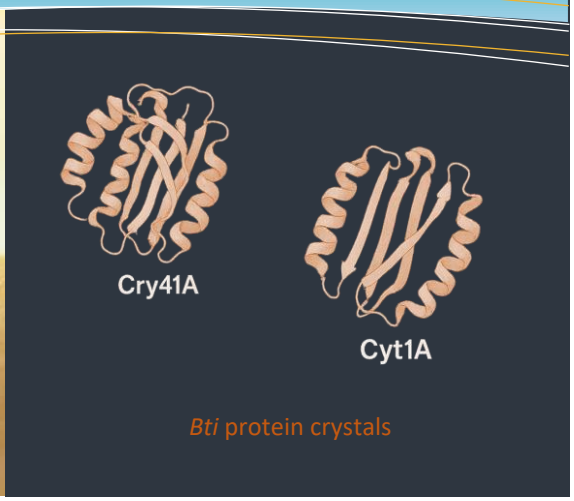
GERMAN SOCIETY OF
TOXICOLOGY

Poison of the month



Bti protein crystals

The proposal to select *Bti* as Toxin of the Month for July 2025 came from the Working Group on Biogenic Toxins…

…to highlight the diverse mechanisms of action and application areas of biogenic toxins, which on the one hand offer great potential for targeted pest control but on the other hand may also pose underestimated ecotoxicological risks. *Bti* exemplifies the need for differentiated, evidence-based evaluation of naturally occurring toxins.

Bti

is a specific subspecies of Bacillus thuringiensis and a spore-forming, gram-positive soil bacterium that synthesizes crystalline δ-endotoxins during sporulation.

The crystals are solubilized in the highly alkaline gut environment (pH > 10) of mosquito larvae. Additionally, larval proteases activate the protoxins into active toxins. These bind highly specifically to cadherin, aminopeptidase, and alkaline phosphatase receptors of the epithelial cells. This binding leads to oligomerization of the toxins and pore formation, resulting in uncontrolled ion transport, cytolysis, and gut barrier disruption.

Bti: Selective Biolarvicide – At the Intersection of Effectiveness and Biodiversity

Bacillus thuringiensis israelensis (Bti) is a naturally occurring soil bacterium that has been deliberately deployed since the 1980s for the control of mosquitoes (*Culicidae*), blackflies *(Simuliidae*), and other blood-feeding insects. Particularly in floodplain areas along the Rhine River, *Bti* has been applied extensively for decades to suppress the mass proliferation of mosquito larvae and thereby reduce the nuisance caused to the population by the so-called “Rhine mosquitoes.”

During sporulation, the bacterium produces protein crystals (Cry and Cyt toxins) that are highly toxic to mosquito larvae. Following oral ingestion by the larvae, these toxins are activated in the gut and destroy the intestinal epithelium through multiple mechanisms. The resulting disruption of the gut barrier leads to uncontrolled ion and water flux, culminating in osmotic shock and sepsis, which kill the larvae within 24 hours.

The specificity of these toxins arises from their compatibility with receptors present only in target organisms. Vertebrates lack homologous gut receptors. To date, studies have demonstrated no acute toxicity in warm-blooded animals (NOAEL > 5000 mg/kg body weight in rat models) following oral, inhalational, or dermal exposure. Moreover, there is no evidence of carcinogenic, mutagenic, or reproductive toxicity. *Bti* is also considered largely harmless to fish, mollusks, and benthic macroinvertebrates, although additive effects from repeated long-term applications cannot be fully excluded. The World Health Organization classifies *Bti* in toxicity category U (“unlikely to present acute hazard”). Although *Bti* is regarded as selective and safe under current knowledge,

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adverse effects on the environment and non-target organisms cannot be entirely ruled out. Several studies indicate that repeated applications of *Bti* may negatively affect other aquatic insects, such as chironomids (non-biting midges), as well as amphibian larvae. Given that *Bti* is employed not only for mosquito control but also globally in biological pest management in agriculture, forestry, and horticulture—targeting pests such as fungus gnats and oak processionary moths—it is of critical importance to detect any potential impacts on biodiversity in sensitive ecosystems at an early stage.

Bti has been approved in the European Union since 2013 as a biocidal active substance for insecticides (Product Type 18). The formulations used contain *Bti* spores and crystal proteins in standardized concentrations and are predominantly applied as granules, either by helicopter or by hand. To date, no harmonized occupational exposure limits for *Bti* spores have been established. While inhalational and oral exposure to *Bti*-containing products is generally considered safe for humans, the protective measures specified in product safety data sheets—such as respiratory protection, gloves, and safety goggles—must nevertheless be observed during application to prevent respiratory irritation and potential sensitization.

A new regulation introduced in 2024 stipulates that *Bti*-containing products may only be applied by professionally trained personnel. Consequently, distribution to the general public, which was previously common practice in affected areas, is no longer permitted. This change is based on a decision by the EU Standing Committee on Biocidal Products (SCBP) and is intended to mitigate potential risks to ecosystems and non-target organisms arising from improper use.

By Ute Haßmann

Authorization

The approval of *Bti* is currently limited until December 31, 2028, and is subject to mandatory re-evaluation under Regulation (EU) No. 528/2012 to decide on an extension based on current toxicological and ecotoxicological findings. The German Federal Institute for Occupational Safety and Health (BAuA) and the European Chemicals Agency (ECHA) coordinate the risk assessment.

KABS

Along the Upper Rhine, *Bti* is mainly applied by the Municipal Action Group for Mosquito Control (KABS), which relies on scientific monitoring and control to maintain a “mosquito-poor yet natural” region.
Before the introduction of *Bti,* various chemical larvicides with significant ecotoxicological drawbacks were used in the Rhine area: as early as the 1920s, oils and paraffins were applied, harming aquatic fauna, amphibians, and birds. Until the late 1970s, organophosphates such as malathion and fenitrothion were used, which are highly neurotoxic and bioaccumulative. Temephos remained in use into the 1980s but proved more persistent in sediments than expected. The switch to *Bti* was made because of its selectivity and rapid degradation, even though non-target insects such as chironomids are adversely affected.

Literature and links:

- KABS: Grundlagen der Mückenbekämpfung
  https://www.kabsev.de/bekaempfung/grundlagen/
- WHO: Bacillus thuringiensis: environmental and health effects, 1999
  Documents\EHCs\EHC-217\EHC217.PDF
- OECD: Series on Pesticides No. 45: Data on Bti
  https://www.oecd.org/env/ehs/pesticides-biocides/seriesonpesticidespublications.htm
- EPA: Reregistration Eligibility Decision (RED) for Bacillus thuringiensis, 1998
  https://archive.epa.gov/pesticides/reregistration/web/pdf/0247red.pdf
- Baumann, L., Baumann, P. Current Microbiology 23, 51–57 (1991).
- Effects of components of theBacillus sphaericus toxin on mosquito larvae
- UBA: Biozide – Risikobewertung Bti
  Biozide | Umweltbundesamt
- Änderung an der ChemBiozidDV wirkt sich auf Bti-Tabletten aus - KABS e.V.
- Foto von National Institute of Allergy and Infectious Diseases auf Unsplash