Ministry of Environment and Food of Denmark
Environmental Protection Agency

Risk Assessment of *Bacillus thuringiensis* subsp. *kurstaki* strains for renewal in EU

Birte Fonnesbech Vogel, Ph.D.
Senior Scientific Advisor

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Introduction

- ***Bacillus thuringiensis subsp. kurstaki*** **strains ABTS 351, PB 54, SA-11, SA-12, EG2348 approved under Regulation (EC) No 1107/2009 ((EU) No 540/2011, (EU) No 541/2011)**
- **RMS DK have submitted the RAR's of the five Btk strains**
- **EFSA Pesticide Peer Review Meeting TC 25 (02-06 March 2020)**

Threshold of 10^5 CFU/g –
risk of food-borne poisonings caused by the *B. cereus* group

RMS, co-RMS and some MSs disagreed.

1. **Comments on the Scientific Opinion from EFSA (BIOHAZ) 2016**
2. **RMS risk assessment for renewal of *Btk* strains**

Scientific Opinion from EFSA (BIOHAZ) 2016

"Risks for public health related to the presence of *Bacillus cereus* and other *Bacillus* spp. including *Bacillus thuringiensis* in foodstuffs"

- **Update of "Opinion of the Scientific Panel on Biological Hazards on *Bacillus cereus* and other *Bacillus* spp in foodstuffs.** *The EFSA Journal* (2005) 175, 1-48
- **Focus on risk and possibility to identify *Bt* in foodstuffs**
- **Evaluation alleged food-borne outbreak in Germany (2012)**

Thorough assessment, revision and new information

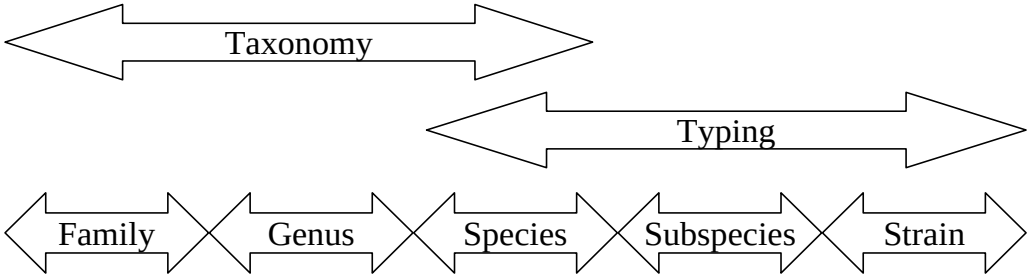
EFSA's role is restricted to risk assessment!

Scientific Opinion from EFSA (BIOHAZ) 2016

Distinction between *Bt* in general and approved biopesticidal *Bt* strains:

"The levels of B. cereus group posing a health risk to consumers are highly strain-dependent due to the highly diverse pathogenic potential (p. 41; 3.5.3)"!!!

- **No new information to question previous risk assessments of approved biopesticidal *Bt* strains**
- **Theoretical possibility: some *Bt* strains capable of causing enterotoxin production in the gut => adverse effects**
- **No evidence implicating approved biopesticidal *Bt* strains**



Scientific Opinion from EFSA (BIOHAZ) 2016

Current evidence implicating biopesticidal *Bt* strain:

1. **Jackson et al. 1995: 18 individuals, nausea and vomiting! Norovirus present in one and *Bt* in 5 stool sample, isolated strains able to produce enterotoxin *in vitro***

2. **McIntyre et al. 2008: *Bt* linked to 4 out of 39 food-borne outbreaks 1991-2005, 62 & 85% - nausea and vomiting! *Bt* only retrieved from food**

3. **German outbreak 2012: 3 out of 5 fell ill 1 a.m., eaten lettuce and Spätzle in the evening, nausea, diarrhea and vomiting!, *Bta* present in lettuce, *Bc* present in Spätzle, "*it is not clear if those persons who consumed the salad also ate cheese noodles*"!!!**

Lack of evidence to implicate approved *Bt* strains as the causative agent!

Approved *Bt* strains have a longstanding proven track record for safety!

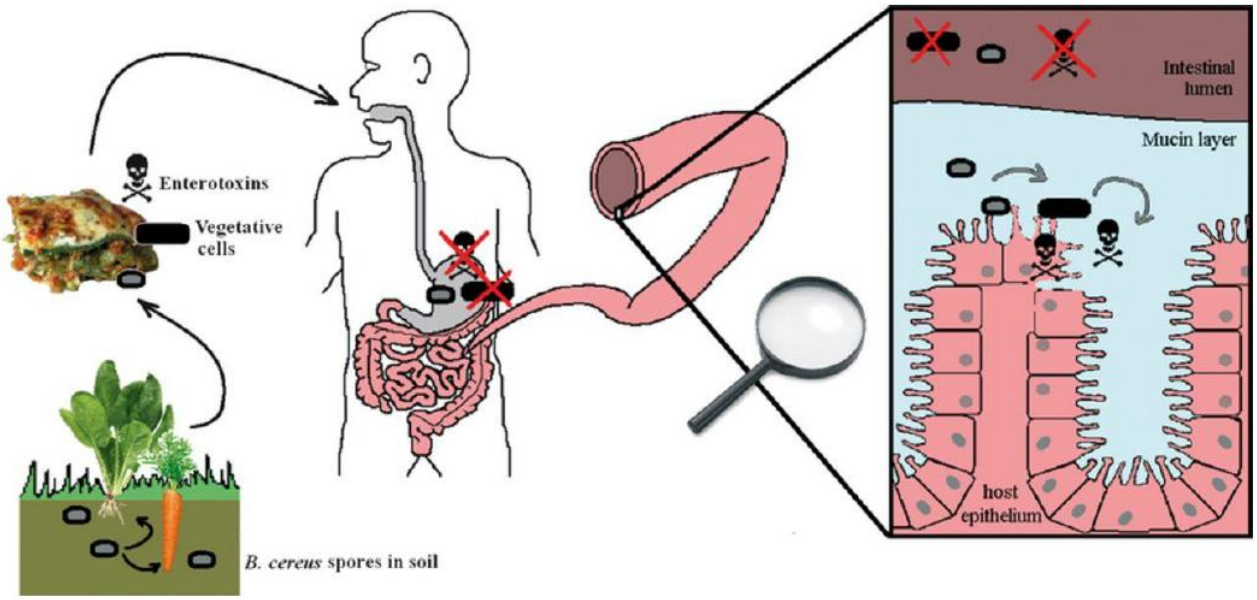
Risk Assessemnt - absence of coding genes for toxins of *Btk* strains

Toxin	Biological activity	Remarks	Produced by some *B. thuringiensis* strains	Produced by commercial Btk strains
Cereulide	Emetic syndrome	-	No	No
ß-exotoxin	Pulmonary toxicity	-	Yes	No
Enterotoxins				
Non-hemolytic enterotoxin	Cytolytic activity against erythrocytes and epithelian cells, Diarrheal type of B. cereus food poisoning	Three components, biological activity requires at least two or even all three components which bind sequential to the host cell	Yes	Yes
Hemolysin	Hemolytic and dermonecrotic activity	Involved in non-GI infections	Yes	Yes
PlcR	Global regulator for transcription of enterotoxin genes	-	Yes	Yes
Cyt K	Pore forming toxin, severe necrotic enteritis (CytK1 only)	Two variants, CytK1 /CytK2, CytK1 highly cytotoxic, CtyK2 not involved in enterotoxicity	CytK1: no CytK2: yes	CytK1: No CytK2: Yes
Other virulence factors				
Sphingomyelinase	Synergistic interaction with NHE and HBL, described symptoms in patients: sepsis and endophtalmitis	Lethal to mice	Observed in low concentrations in very few strains[#]	No
Haemolysin II	Apoptosis in macrophages	-	Not yet observed	No
InhA1	Escaping of B. cereus from macrophages	-	Not yet observed	No
NprA	Metalloprotease, immune evasion and tissue degradation by B. anthracis	-	Not yet observed	No

The presence of a certain enterotoxin gene does not mean that it will be expressed

Risk Assessment - virulence factors

Conditions for Bt strains survival, germination and enterotoxin production in the human gut



The figure show hypotheses of the course of *Bacillus cereus* diarrhoeal food poisoning. Reproduced from Ceuppens *et al*., 2013.

For a Bacillus cereus group strain to cause a diarrheal event spores must:

1. **survive digestion,**
2. **adhere to intestinal lining,**
3. **germinate,**
4. **and then produce a relevant amount of toxin.**

Risk Assessment – Physiological differences

Commercial Btk strains differ from pathogenic *B. cereus* strains with regard to their physiology:

- **germination in general, restricted to conditions in the host insect gut** (Du & Nickerson, 1996; Abdoharrahem *et al*., 2009, King *et al*., 2012)
- **have lower growth rates than pathogenic *B. cereus* strains** (Hansen *et al*., 2011)
- **grow less well at high temperatures** (Hansen *et al*., 2011)
- **grow less well at microaerobic conditions** (Hansen *et al*., 2011)
- **pathogenic *B. cereus* strains might be better adapted to survival in the human body than commercial Bt strains** (Hansen *et al*., 2011)

Fotos by Magnus Gammelgård

Risk Assessment – Pathogenic Potential

Commercial *Btk* strains have a much lower toxigenic potential than pathogenic *B. cereus* strains:

- **Pathogenic *B. cereus* strains have a higher potential to adhere to living surfaces** (Auger *et al*., 2009)
- **Pathogenic *B. cereus* strains produce much higher amounts of enterotoxins under optimal laboratory conditions than commercial Bt strains do** (Damgaard, 1995; Confidential RARs of 4 Btk strains)
- **Reduced competitive ability and infectious potential in the human gut due to plasmid-encoded high expression of Cry toxins in biopesticidal *Btk* strains** (Raymond and Federici, 2010)
- **Commercial *Bt* strains are not able to produce the emetic toxin cereulide** (Kim *et al*., 2015; EFSA Scientific Opinion, 2016; RARs of 4 Btk strains)
- **No toxicity, pathogenicity or infectivity was noted in experimental animal studies with approved *Btk* strains** (RARs of 5 Btk strains)

Risk Assessment - Conclusion

- **Half century of commercial use => Lack of evidence to implicate approved *Bt* strains as the causative agent**

- **Strain specificity is central to determine the potential for pathogenicity**
  - **Specific identification methods needed**

- **Commercial *Btk* strains**
  - **differ from pathogenic *B. cereus* strains with regard to their physiology**
  - **have a much lower toxigenic potential than pathogenic B. cereus strains**

- **It is unlikely that biopesticidal *Btk* strains are able to produce enterotoxin at biologically relevant levels**
  - **Plasmid-encoded high expression of Cry toxins in biopesticidal *Btk* strains is very likely to reduce competitive ability and infectious potential in the human gut**

Prediction of a safety level for commercial *Btk* strains based on information of pathogenic *B. cereus* isolates is not justified.

~~**Threshold of 10^5 CFU/g –**~~
~~**risk of food-borne poisonings caused by the *B. cereus* group**~~

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Thank you for your attention!

Birte Fonnesbech Vogel
Ph.D. Senior Scientific Advisor
Environmental Protection Agency
Pesticides and Biocides
Tolderlundsvej 5 , 5000 Odense
Denmark
bifvo@mst.dk

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