European Commission

Draft Renewal Assessment Report prepared according to the Commission Regulation (EU) N° 1107/2009

Microbial Pest Control Agent (MPCA)
Bacillus thuringiensis
subsp. *kurstaki* SA-12

Volume 3 B.6 (MPCA)
Effect on human health

Rapporteur Member State: Denmark

Co- Rapporteur Member State: The Netherlands

Version history

When	What
2008	DAR
2011	Addendum to the DAR
2018	Initial RAR

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B Summary of the data and information

B.6 Effects on human health

INTRODUCTION

Bacillus thuringiensis subsp. *kurstaki* SA-12 (in the following abbreviated as Btk SA-12) was one of the existing active substances covered by the Regulation (EC) No 2229/2004 on the implementation of the fourth stage of the program of work referred to in Article 8(2) of Council Directive 91/414/EEC. In Annex I to Regulation (EC) No 2229/2004 the Commission designated Denmark as rapporteur Member State to carry out the assessment of Btk SA-12 on the basis of a joint dossier submitted for the Btk strains SA-11, SA-12 and EG 2348. The notifier for Btk SA-11 and SA-12 was Mitsui AgriScience International SA/NV while EG 2348 was notified by Mitsui AgriScience International SA/NV and Intrachem Bio Italia S.p.A. (now CBC (Europe) S.r.l.). In accordance with the provisions of Article 22(1) of Regulation (EC) No 2229/2004, Denmark submitted in January and February 2008 to the EFSA the draft assessment report, including, as required, a recommendation concerning the possible inclusion of Btk SA-12 in Annex I to the Directive. The Commission examined the draft assessment report, the recommendations by the rapporteur Member State and the comments received from other Member States in consultation with experts from a certain number of Member States. The Commission referred on 12 July 2008 a draft review report to the Standing Committee on the Food Chain and Animal Health, for final examination. The draft review report was finalized in the meeting of the Standing Committee on 12 July 2008. Subsequently Regulation (EC) No 1107/2009 repealed and replaced Directive 91/414/EEC and the active substance Btk SA-12, was deemed to be approved under that Regulation and included in the Annex to Regulation (EC) No 540/2011. EFSA delivered its conclusions on *Bacillus thuringiensis* ssp. *kurstaki* (strains ABTS-351, PB-54, SA-11, SA-12, EG2348) on the 16 December 2011 (published 23 February 2012). Based on this new information available, no need to change the conditions of approval of Btk SA-12 was identified. The Commission filed on 13 December 2013 an updated review report for Btk strains SA-11, SA-12 and EG 2348 to the Standing Committee on the Food Chain and Animal Health for examination.

The approval of Btk SA-12 under the Regulation (EC) No 1107/2009 expires 30 April 2019. In accordance with the same Regulation the original notifier Mitsui AgriScience International SA/NV has filed to the Commission an application for the renewal of the approval of the active substance Btk SA-12 on 30 April 2016. In accordance with Regulation (EU) 2016/183 the notifier submitted to the designated RMS Denmark, the co-RMS The Netherlands as well as to EFSA and Commission a dossier for renewal of Btk SA-12 considering the deadline stated in SANTE-2016-10616–rev. 3.

Btk SA-12 is a wild type strain originating from infested insects. Btk acts highly specific against insect species of the order Lepidoptera and is not expected to have any harmful effects on beneficials and other non-target species of other insect orders. The insecticidal activity of Btk is mainly attributed to spore bound insecticidal pro-proteins (*Cry* toxins) which are ingested by the target pests and activated under alkaline conditions in the midgut of the larvae. The first assessment of the strain proved that it does not have any harmful effects on human or animal health or on groundwater or any unacceptable influence on the environment. The overall conclusion from EFSA (2012) confirms that no critical areas of concern are identified within the framework of the use which was supported.

As the manufacturing process of Btk SA-12 has not been changed since original approval, all data submitted for the original approval of the strain are considered fully applicable for the current evaluation.

For the renewal of the Btk strains SA-11, SA-12 and EG 2348 under Regulation (EC) 1107/2009, a separate dossier was submitted for each strain only including data, which have previously not been submitted or evaluated. Nevertheless, there is some information which is applicable to all three Btk strains, e.g. published information for Btk in general obtained during searches for peer reviewed literature according to EFSA Guidance (2011)[1] carried out for relevant sections.

In the following for ease of information, full study summaries/sections taken from the DAR (2008) or its Final Addendum (2011) are included if they are considered relevant for renewal of Btk SA-12. In order to facilitate discrimination between new data and data already evaluated during the first approval process, the headline "New Data" begins the section with data, which have previously not been submitted or evaluated. Data and their evaluations from the original DAR and addenda to the DAR are highlighted by grey background.

1 Guidance of EFSA: Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009. EFSA Journal 2011;9(2):2092

B.6.1 Tier I

B.6.1.1 Basic information

Bacillus thuringiensis has been used for over 60 years for insect pest control, and there have only been a few reports of clinical infections caused by this organism. However, cases of human illness may have been under-reported because of the close relationship to *B. cereus*, and in clinical settings there is no discrimination between the two species. Strains of *B. thuringiensis*, including the commercial ones, produce the same enterotoxins known from *B. cereus* to cause diarrhoea in humans (Glare & O'Callaghan, 2000), still the significance of *B. thuringiensis* as a causative agent for human disease is not known (WHO, 1999).

Diarrhoea is induced by enterotoxins produced in the small intestine by growth of ingested *B. cereus* at levels exceeding 10^5 CFU/g (EFSA, 2005)[2]. *B. cereus* has been identified in only 1-2% of food borne outbreaks in Europe (EFSA, 2005), but this number may be under-estimated since reporting is not mandatory and the poisoning is usually mild and transient, although rare fatal cases have been reported.

Several animal studies have shown safety of *B. thuringiensis* (Siegel, 2001; McClintock et al., 1995), however it is unclear whether these studies can be extrapolated to humans, since at least for predicting the diarrhoeal potential of *B. cereus* or *B. thuringiensis*, no useful animal models are available (Wilcks et al., 2006b). On the other hand, with the exception of ocular and dermal irritation case reports, no adverse health effects have been recognized after occupational exposure to plant protection products based on *B. thuringiensis* (WHO, 1999).

When assessing the toxicity to humans of *B. thuringiensis* it is necessary to test each specific strain since there is large variation in the genus, and it is not possible to extrapolate from one species to another. When e.g. testing *B. thuringiensis* strains in rabbit ileal loop studies, researchers found strains both negative and positive in the test (Itoh et al., 1991). Even the same strain showed different results, indicating that some host specific factors also influence the virulence of the strain, and that there may be a risk for immunocompromised persons.

However, since SA-11, SA-12 and EG2348 cannot be discriminated on chromosomal level (refer to Identity part, B1), they are believed to act similar in mammalian toxicology and pathogenicity, since so far human pathogenic traits in *B. thuringiensis* have been primarily located to the chromosome.

Evaluation RMS	Relevant information has already been submitted for first approval of Btk SA-12. The review report for *Bacillus thuringiensis* subsp. *kurstaki*, strains SA-11, SA-12 and EG 2348[3], as well as the EFSA Conclusion[4] indicated no harmful effects on human or animal health by Btk SA-12.

New information

B. cereus and *B. thuringiensis* strains are very similar and, consequently, are not usually distinguished in cases of food poisoning using routine methodology. The EFSA Panel on Biological Hazards (BIOHAZ)[5] has recently concluded that the levels of *B. cereus* considered to be a consumer risk for are $>10^5$ organisms/g food (although this will be strain-specific). However, cases of both emetic and diarrhoeal illness have been reported involving lower levels of *B. cereus*. The levels of *B. cereus* that can be considered as a risk for consumers are also valid for *B. thuringiensis*. There is no evidence that *B. thuringiensis* has the genetic determinants for the emetic toxin cereulide.

EFSA further recommend the use of genome sequencing to discriminate *B. cereus* from *B. thuringiensis* in food poisoning cases (EFSA, 2016). *B. cereus* diarrhoeal syndrome has a typical incubation time of 8-16 hours and is associated with gastrointestinal pain and watery diarrhoea, and is attributed to the action of the enterotoxins haemolysin BL (hBL), non-haemolytic enterotoxin (Nhe) and cytotoxin K (CytK). Enterotoxin is degraded by heating and by stomach enzymes; its action may therefore be due to toxin production in the gastrointestinal tract. *B. cereus* emetic syndrome is associated with nausea and vomiting and has a typical incubation time of 1-5 hours. Symptoms are caused by the heat-stable toxin cereulide.

[2] EFSA Panel on Biological Hazards (BIOHAZ). Opinion of the Scientific Panel on Biological Hazards on Bacillus cereus and other Bacillus spp in foodstuffs. The EFSA Journal (2005) 175, 1-48.
[3] European Commission, 2008. Review Report for the active substance *Bacillus thuringiensis* subsp. *kurstaki* (strains SA 11, SA 12, EG 2348), SANCO/1543/08 – rev. 4, 13.12.2013
[4] European Food Safety Authority; Conclusion on the peer review of the pesticide risk assessment of the active substance *Bacillus thuringiensis* ssp. *kurstaki* (strains ABTS 351, PB 54, SA 11, SA 12, EG 2348). EFSA Journal 2012; 10(2):2540.
[5] EFSA Panel on Biological Hazards (BIOHAZ). Risks for public health related to the presence of *Bacillus cereus* and other *Bacillus* spp. including *Bacillus thuringiensis* in foodstuffs. EFSA Journal 2016;14(7):4524-4516.

Only two papers, besides the alleged food poisoning outbreak described in the background section of BIOHAZ Opinion, report on the involvement of *B. thuringiensis* in human outbreaks. The first is a description of one outbreak, while the other is a reassessment of isolates from 39 outbreaks. In this paper, it is suggested that *B. thuringiensis* might have been the cause of the infection in four of the outbreaks. In the alleged food poisoning outbreak, it appears that the only bacteria that were found above the generally accepted level were *B. cereus* group bacteria, identified as *B. thuringiensis* in the salad samples. The *B. thuringiensis* isolated from the salad were characterised by FTIR spectroscopy and could not be discriminated from *B. thuringiensis* subsp. *aizawai* (XenTari) which had been sprayed on the salad on the field. The leftovers of cheese noodles were also tested for the presence of *B. cereus*, with levels reported as 6 x 10^3 CFU/g. It is not clear if those persons who consumed the salad also ate cheese noodles. Consequently, there is very little evidence to suggest that residues of *B. thuringiensis* as a species on foodstuff may cause illness, and even less to implicate approved bio-pesticide strains.

A literature search according to EFSA Guidance (2011)[6] was conducted in May 2016 covering the last 10 years. The literature research was carried out on the search-engine from the German Institute of Medical Documentation and Information – DIMDI (http://www.dimdi.de/static/en/index.html) and comprised the bibliographic databases MEDLINE, BIOSIS, CAB, and SCISEARCH. The search strategy aimed to find all recent (from 2006 onwards) references that are of toxicological relevance, regarding possible effects of *Bacillus thuringiensis* subsp. *kurstaki*. After rapid assessment based on title and abstract; 13 references were submitted to a detailed assessment of full text documents. In total, 11 references were considered relevant and reliable and are summarised under the respective data points below. For more details please refer to Seehase (2016) in point B.6.3 below. Data requirements for metabolites of Btk are also covered by the literature search. Btks´ insecticidal proteins were shown to be of no concern for human health. Please refer to Vol. 3 MA, Point B.2.8. Additionally, the close relationship and the toxigenic potential of Btk due to possible production of *B. cereus* enterotoxins is in detailed discussed in Vol. 3 MA, Points B.2.6 and B.2.8. References which provide data on toxicity or an endpoint, even if a species or strain different from strain of interest or non-model species were used, are summarized in extended format including details on material and methods of the study. References which provide only supporting information are summarized shortly within the summary text and the abstracts are presented at the end of each data point.

Laboratory studies on mammalian toxicity of Btk SA-12 indicate very little safety risk from direct exposure (see also Table 6.4.1-1). No adverse effect and clearance of spores within 7 days was observed upon oral or pulmonary dosing. Only extremely high dose levels of 9×10^{10} CFU/mL per animal applied intraperitoneally caused mortality in mice while no signs of toxicity were noted at dose levels of 10^7 CFU or lower (Berlitz et al. 2012).

Although Btk is capable of producing diarrhoeal enterotoxins no diarrhoea or serious health issues have been observed in experimental rodent studies at high dose levels of Btk (Wilcks et al. 2006a). Furthermore, epidemiological studies conducted during aerial spraying campaigns with Btk pesticides or occupational health reports from production plants of Btk have not reported a significant increase in diarrhoeal symptoms in Btk-exposed residents, operators, or workers (Hansen et al., 2010, Levin, 2009). Thus, there is no valid evidence linking Btk with episodes of diarrhoea.

A study on greenhouses workers exposed to Btk-based MPCPs revealed that the large amount of naturally occurring airborne microorganisms is supposed to have a greater influence on the workers´ health than applied microorganisms from MPCP (Hansen et al., 2010).

Even though an increase in humoral antibodies towards Btk was detected upon occupational exposure, no adverse health effects were noted including no effects on respiratory symptoms or lung function. Moreover, prevalence rate ratios among exposed individuals did not increase significantly over a 3-year period (Baelum et al., 2012).

No toxicity or infectivity was noted in experimental studies with approved Btk[7] strains upon oral, inhalative, or intravenous exposure even to exceedingly high dose levels. Taking together the results of these experimental studies, epidemiological and occupational evidence and the experience from several decades of safe application of Btk-based plant protection products it is appropriate to state that there is no concern with regard to human health.

Evaluation RMS	The opinion by EFSA (BIOHAZ) represents a thorough update of the previous EFSA opinion on *Bacillus cereus* and other *Bacillus* spp. published in 2005. In the new version specific focus has been placed on current knowledge regarding the risks and possibility to

[6] Guidance of EFSA: Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009. EFSA Journal 2011;9(2):2092

[7] European Food Safety Authority; Conclusion on the peer review of the pesticide risk assessment of the active substance *Bacillus thuringiensis* ssp. *kurstaki* (strains ABTS 351, PB 54, SA 11, SA 12, EG 2348). EFSA Journal 2012; 10(2):2540.

	identify *Bacillus thuringiensis* in foodstuffs, an organism of which specific strains are used extensively world-wide as plant protection products. Additionally, the BIOHAZ panel was requested to specifically consider and evaluate the alleged food-borne outbreak in Germany (2012), in which a salad containing *B. thuringiensis* was suspected to be the source of the outbreak. The RMS finds the EFSA opinion document to represent a very thorough assessment and revision of the topic with much valuable new information. We particularly find it important for scientific risk assessment to consider differences between *B. thuringiensis* at the strain level and thus to make a clear distinction between *B. thuringiensis* in general and the specific strains, approved and used as bio-pesticides. Although this point has been addressed in the new opinion (e.g. "*The levels of B. cereus group posing a health risk to consumers are highly strain-dependent due to the highly diverse pathogenic potential"*) we do not find any new information to question previous risk assessments of approved biopesticidal *B. thuringiensis* strains, which in our opinion have a longstanding proven track record for safety. We do not dispute the theoretical possibility to find *B. thuringiensis* strains in nature, which are capable of causing enterotoxin production in the gut environment in humans with possible adverse effects, however we do not find any convincing evidence implicating approved biopesticidal *B. thuringiensis* strains as being causative agents of foodborne intestinal diseases. It is important to note that the plasmid-encoded high expression of Cry toxins in biopesticidal strains is very likely to reduce their competitive ability and infectious potential in the human gut (Raymond et al. 2017[8]). To our knowledge there is very little evidence to suggest that residues of *B. thuringiensis* as a species on foodstuff may cause illness, and even less to implicate approved biopesticide strains. This is well reflected in the EFSA opinion, which states that: "*Due to lack of available data, it is not possible to conduct a quantitative evaluation of the risk to public health arising from the presence of B. thuringiensis in food. Therefore, only a qualitative evaluation has been done…*" In spite of the widespread use of these strains for pest control, only two scientific investigations are presented in the opinion that connect *B. thuringiensis* to food poisoning apart from the mentioned alleged outbreak in Germany in 2012 discussed below. The first of these investigations reports on an outbreak in a chronic care institution involving 18 individuals of which potential pathogens were only identified in five (Jackson et al. 1995). Predominant symptoms included **nausea and vomiting**, which are, notably, <u>not</u> normally attributed to enterotoxins produced in the gut environment, but are consistent with the fact that Norovirus (popularly known as *winter vomiting bug*), was present in at least one of the four individuals found to have *B. thuringiensis* in stool samples. We find lack of evidence in this study to conclusively implicate *B. thuringiensis*, even though the isolated strains were able to produce enterotoxin under laboratory conditions. It is important to note that <u>*B. thuringiensis* used as bio-pesticides do not encode the emetic toxin</u>, which is attributed to symptoms of nausea and vomiting in *B. cereus* related food poisoning. The second study (McIntyre et al. 2008) showed that *B. thuringiensis* was linked to a total of four out of 39 food-borne outbreaks between 1991 and 2005 in Canada, in which *Bacillus cereus*-like isolates were either found in food or clinical samples. Notably *B. thuringiensis* was not retrieved from any clinical samples (only from food) and furthermore 62% and 85% of the affected individuals indicated **nausea and vomiting** respectively as symptoms. As stated above these symptoms are not caused by enterotoxins putatively produced in the gut environment by *B. thuringiensis*, suggesting that this species was not the causative agent. Again, we find lack of evidence in this study to conclusively implicate *B. thuringiensis* in food-borne outbreaks. Lastly, in the alleged German outbreak the symptoms of the affected people were reported in the BfR Sample Documentation Form to be: "*…**nausea,** abdominal crampings, diarrhea*

[8] Raymond BD, Federici B (2017). In defense of *Bacillus thuringiensis*, the safest and most successful microbial insecticide available to humanity – a response to EFSA. *FEMS Microbiology Ecology.* Volume 93, Issue 7

	*and **vomiting.** Fallen ill at 1 o'clock at night after consuming lettuce and Spätzle (Swabian cheese noodles) in the evening. 5 persons had lettuce and Spätzle, 3 of them fell ill, those who did not fall ill did not have lettuce.*" Again, the fast onset of illness and symptoms including nausea and vomiting are not consistent with enterotoxins produced in the gut environment (by *B. thuringiensis*). In our opinion too little attention has been placed on the fact that *Bacillus cereus* was present in the cheese noodles (6.0×10^3 CFU/g), which due to potential emetic toxin production seems much more consistent with the symptoms observed. Indeed, intoxication by the emetic toxin is most frequently linked to consuming starchy foods (including rice, pasta and noodles) exposed to temperatures allowing preformation of the toxin. In the EFSA opinion the authors' state: "*It is not clear if those persons who consumed the salad also ate cheese noodles…*" This in our opinion is a very central question to ask, and we are surprised that this has not been clarified. In conclusion, it is our opinion that the provided information in this outbreak investigation falls very short of providing enough evidence to implicate approved *B. thuringiensis* as the causative agent of the very limited outbreak in Germany. No toxicity or infectivity was noted in experimental studies with approved Btk strains[6] upon oral, inhalative, or intravenous exposure even to exceedingly high dose levels. Taking together the results of these experimental studies, epidemiological and occupational evidence and the experience from several decades of safe application of Btk-based plant protection products it is appropriate to state that there is no concern with regard to human health.

Cited references:

Report: KMA 6.1.1/07 – Seehase, S. (2016), Literature review on *Bacillus thuringiensis* subsp. *kurstaki* SA-12: Toxicology

Unpublished report

Abstract: not applicable

Evaluation RMS	The literature search was accepted as valid, both regarding inclusion of databases and use of search terms. Please refer to point B.6.3.

Report: KMA 6.1.1/11 – Levin, D.B (2009), Human health effects resulting from exposure to *Bacillus thuringiensis* applied during insect control programs

Published article

Use of Microbes for Control and Eradication of Invasive Arthropods, Volume 6 of the series Progress in Biological Control, pp 291-303

Abstract:

Products based on *Bacillus thuringiensis* (*Bt*) such as Foray 48B, which contains *Bt kurstaki* strain HD-1, must meet rigorous standards required by the US Environmental Protection Agency, the US Food and Drug Administration, the Canadian Pesticide Management and Regulatory Agency, and Health Canada, before they are approved for commercial use in Canada and the US. These agencies consider *Bt*-based products to be neither toxic nor pathogenic to mammals, including humans. Despite these approvals, there remains widespread public concern about negative health effects associated with aerial applications of *Btk* during insect control programmes. Major health impact assessment studies in the US and Canada suggested there were no negative short-term human health effects associated with aerial applications of Foray 48B. A similar health impact assessment conducted in New Zealand reported short term irritant effects and some worsening of pre-existing conditions such as allergies and asthma. These findings warrant further investigation following aerial applications of commercial *Bt* products in populated urban areas.

Evaluation RMS	No remarks

B.6.1.1.1 Medical data

At species level:

A research worker developed a marked local reaction and lymphadenitis following a needle stick injury when handling *B. thuringiensis* subsp. *israelensis*. Besides *B. thuringiensis*, *Acinetobacter calcoaceticus* was isolated from the exudates (Warren et al., 1984) and could have been involved in the reactions.

B. thuringiensis has been isolated from burn wounds in two patients, none of the isolates showed any toxicity to Vero cells (Damgaard et al., 1997). Hernandez et al. (1998) isolated *B. thuringiensis* (subsp. *konkukian*) from a war wound, the strain could afterwards infect immunosuppressed mice after cutaneous infection. Furthermore the strain was lethal for immunocompetent mice after pulmonary infection (Hernandez et al., 1999).

B. thuringiensis was isolated from four individuals during the investigation of a gastroenteritis outbreak in a chronic care institution, the isolates showed the same cytotoxic effects as *B. cereus* (Jackson et al., 1995).

Green et al. (1990) reported that *B. thuringiensis* was isolated from body fluids of 55 patients with different infectious diseases. In most cases it was considered a contaminant and not the cause of illness, but for three patients, *B. thuringiensis* could neither be ruled in nor out as a pathogen. Furthermore, *B. thuringiensis* was isolated from the conjunctiva of a spray worker with conjunctivitis after an accidental splash with a *B. thuringiensis* product.

A recent report showed that *B. thuringiensis* is an important ocular pathogen with *B. thuringiensis* isolated from 26% of the ocular infection cases studied (Callegan et al., 2006).

At subspecies level:

After accidental splashing with a commercial *B. thuringiensis* subsp. *kurstaki* product a farm worker developed a corneal ulcer in one eye (Samples & Buettner, 1983). *B. thuringiensis* was isolated from the infected eye; however, it was not considered whether *B. thuringiensis* might have been a non-pathogenic contaminant of the ulcer.

In addendum to the DAR 2011 these clarification of details were provided:

Hernandez E *et al*., 1999. *Bacillus thuringiensis* serotype H34 isolated from human and insecticidal strains serotypes 3a3b and H14 can lead to death of immunocompetent mice after pulmonary infection. FEMS Immunology and Medical Microbiology 24:43-47.

BALB/c mice were infected intranasally with 10^5-10^8 spores of *Bacillus thuringiensis* subsp. *konkukian* serotype H34, *B. thuringiensis* subsp. *thompsoni* serotype H12, *B. thuringiensis* subsp. *kurstaki* serotype 3a3b or *B. thuringiensis* subsp. *israelensis* serotype H14. Instillation of 10^8 spores of the strains resulted in the following mortalities in the four groups of each five mice: H34 (100%), 3a3b (80%), H14 (40%) and H12 (0 %). When mice were infected with 10^7 spores of H34 no mortality occurred, but only local inflammatory reaction.

Supernatants from a 24 hr stationary-phase culture containing 10^8 CFU/ml was centrifuged and filtered through a 0.22 μm membrane. The filtrate was concentrated and the haemolytic activity corresponding to the fraction higher than 30 kDa was tested. The last dilution titers giving 100% haemolysis were the following for the strains tested: H34 (1/256), 3a3b (1/128), H14 (1/32) and H12 (1/2). Furthermore nasal installation of the concentrated supernatant resulted in killing of all mice (five) after 30 min for the H34 strain and in less than 5 hr for the 3a3b strain. The fractions under 30 kDa were not haemolytic and were not able to kill the mice.

RMS comment: The study shows the potential of some *B. thuringiensis* strains, including subsp. *kurstaki* serotype 3a3b, to cause mortality in mice when administered intranasally at very high concentration. The mortality is probably caused, not by a toxic or pathogenic effect, but is most probable due to an acute allergic reaction. The risk assessment has already pointed out the sensitization potential of the strain, and the requirement to wear personal protection equipment when handling the strain/product at high concentrations.

Callegan MC et al., 2006. Virulence factor profiles and antimicrobial susceptibilities of ocular *Bacillus* isolates. Current Eye Research 31:693-702.

Thirty-nine isolates from eye infections caused by *Bacillus* were investigated. It was found that in 26% of the cases *B. thuringiensis* could be isolated from the ocular infection cases. The identification of *B. thuringiensis* was based on detection of a parasporal crystal by phase contract microscopy. However, there is no information on which serotypes the identified *B. thuringiensis* strains belongs to.

RMS comment: The study shows the potential of strains of *B. thuringiensis* to be involved in ocular infections. However, for *B. cereus* to cause a severe ocular infection, the eye has to be severely injured for the bacteria to

enter the eye. So for the healthy eye no severe infections by *B. thuringiensis* are envisaged, and therefore this point is not relevant for the risk assessment.

Callegan MC et al., 2005. *Bacillus* endophthalmitis: roles of bacterial toxins and motility during infection. Investigative Ophthalmology & Visual Science 46:3233-3238.

The midvitreous part of rabbit eyes were injected with a wild type strain of *B. thuringiensis* subsp. *israelensis* and two mutant strains inactivated in motility and both motility and quorum sensing, respectively. Quorum sensing controlled toxins are essential to virulence during infection. All three strains were able to replicate in the eye, but only the wild type strain was able to migrate into the anterior chamber. A significant difference between the strains was observed, with infection by the wild type strain being much more rapid and severe.

RMS comment: The strains used were subsp. *israelensis* and not subsp. *kurstaki*. Furthermore the infection was caused by injecting the strain directly into the midvitreous part of the eye. For risk assessment this is not considered a relevant exposure route for the healthy eyes of humans.

New data 2016

From the recent literature search, no references were identified, reporting medical cases of Btk SA-12.

Evaluation RMS	Relevant data have already been submitted during first approval of Btk SA-12 and are considered acceptable to cover current requirements. No evidence of a subgroup of individuals who are more sensitive than the majority of the general public to Btk has been documented. No adverse effects were observed in humans exposed to Btk during/after agricultural spraying campaigns performed in Canada, New Zealand, Denmark or USA. There are no case reports linking agricultural use of Btk with human infections although Btk products have been used worldwide for more than sixty years. Moreover, only one reported case of gastroenteritis and one report for corneal ulcer have been reported. However, in none of the cases it was confirmed that the Btk was the causative agent.

B.6.1.1.2 Medical surveillance on manufacturing plant personnel

Statement on the health of personnel working (production, development and manufacture) with *B. thuringiensis* derived products is available from the applicants (Dively, 2005). *B. thuringiensis* derived products have been produced at the manufacturing plant since 1960. Annual physical exams are performed and showed in 1988 that in 30 employees with an exposure history of 3 to 28 years, no health related or adverse reactions were found.

New data 2016

A new health surveillance report is submitted for renewal of Btk SA-12 under Regulation (EC) No 1107/2009 by the manufacturer. No incidents related to adverse health effects to employees, resulting from exposure to *Bacillus thuringiensis* during production, formulation, and handling of microbial products have been reported (Doak 2016). There are employees working for 30 years in the manufacturing plant and no adverse effect have ever been noted.

In the literature search covering the last 10 years and focussing on toxicity or pathogenicity of Btk on mammals, one article was identified concerning medical surveillance on manufacturing plant personnel.

Baelum et al. (2012) evaluated the health effects of exposure to microbiological control agents used in Danish greenhouses including MPCP containing *Bacillus thuringiensis* subsp. *kurstaki* as well *Verticillium lecanii*, and *Trichoderma harzianum.* IgE levels were above the detection limit in 53% of the blood samples of exposed workers. The measurement was, however, only qualitative and no differences between exposed and not exposed samples are detectable. Thus, IgE levels and exposure levels do not correlate and no significant changes in respiratory symptoms, lung function or bronchial hyper-responsiveness were detectable. Additionally, prevalence rate ratios among exposed increased only marginally from 1.20 (CI95%: 1.01-1.42) to 1.43 (CI95%: 1.09-1.87) over a 3-year period.

No health-related reactions were observed in personnel working with Btk-derived products for several years, thus, there is no evidence that Btk may cause serious health effects after repeated inhalatory exposure in humans.

Evaluation RMS	Despite long-term exposure to Btk SA-12 of plant personnel, there was no evidence of any infection, toxicity or pathogenicity of the strain to workers referred to data provided for first approval of Btk SA-12. Present information confirm that no health related reactions have been observed in personnel working with Btk-derived products for several years. Thus, there is no evidence that Btk may cause serious health effects after repeated inhalatory exposure in humans. It has been suggested the use of *B. thuringiensis* in greenhouses may give rise to sensitization because increased IgE against *B. thuringiensis* was seen in 53% of the samples from growers over a 3-year period (Baelum et al. 2012). Despite increased IgE values to Bt which may be regarded as a sign of sensitization, neither prevalence nor incidence of respiratory symptoms was detectable. However, in another study it was shown that the already present airborne bacteria in greenhouses might have a greater influence on growers' health than applied biocontrol strains (Hansen et al., 2010). Overall there is no evidence in the scientific literature that bacteria including *B. thuringiensis* in greenhouses may give rise to sensitization.
Endpoint: Medical data: (including medical surveillance on manufacturing plant personnel)	No incidents related to adverse health effects such as toxicological effects, allergic response, or irritation, to employees, resulting from exposure to *B. thuringiensis* subsp. *kurstaki* SA-12 during production and packaging of the product have been reported

Cited references:

Report: KMA 6.1.1.2/02 – Doak, B. (2016), BTZ Medical Verification

Unpublished report

Abstract: not applicable

Evaluation RMS	No remarks

Report: KMA 6.1.1.2/03 – Baelum J., Larsen P., Doekes G., Sigsgaard T. (2012), Health effects of selected microbiological control agents. A 3-year follow-up study

Published report

Ann Agric Environ Med., 19(4):631-636

Abstract:

INTRODUCTION AND OBJECTIVES: Microbiological control agents (MBCA) are widely used in greenhouses, replacing chemical pesticides. The presented study aims to describe health effects of exposure to three types commonly used: *Bacillus thuringiensis*, *Verticillium lecanii*, and *Trichoderma harzianum* covering seven different products in greenhouse workers with emphasis on sensitization and respiratory effects.

METHODS: 579 persons aged 17 - 67 years culturing ornamental flowers were included. They were followed for three years with annual examinations including interview about exposure and symptoms, lung function, including bronchial (histamine) challenge test, and blood samples. Direct and indirect exposure for each person and year was estimated by information from respondents and employers. IgE in serum against the 7 products of MCBA was analyzed using an enzyme immunoassay technique.

RESULTS: 65%, 40%, and 78% were exposed to *B. thuringiensis*, *V. lecanii*, and *T. harzianum*, respectively, while 6, 3 and 3% were handling the products. IgE against *B. thuringiensis* was seen in 53% of the samples and with prevalence rate ratios among exposed increasing from 1.20 (CI95%: 1.01-1.42) to 1.43 (CI95%: 1.09-1.87) over the 3-year period. There was no relation between exposure to any MBCA and neither prevalence nor incidence of respiratory symptoms and there was no effect on lung function or bronchial responsiveness.

CONCLUSIONS: Use of *B. thuringiensis* in greenhouses may give rise to sensitization while no effect on the occurrence of respiratory symptoms or lung function was observed. The persons had a relatively long exposure. Therefore, a healthy worker effect may have influenced the results.

Evaluation RMS	Increased IgE against *B. thuringiensis* was seen in 53% of the samples from growers over a 3-year period (Baelum et al. 2012). Despite increased IgE values to Bt which may be regarded as a sign of sensitization, neither prevalence nor incidence of respiratory symptoms was detectable. Therefore, it cannot be concluded that use of *B. thuringiensis* in greenhouses may give rise to sensitization.

B.6.1.1.3 Sensitisation/allergenicity observations, if appropriate

Bernstein et al. (1999) observed farm workers (vegetable harvesters) before and after exposure to *B. thuringiensis* subsp. *kurstaki* SA-11 (Delfin). No evidence of occupationally related respiratory syndromes was observed. Positive skin test responses to spore extracts of SA-11 were seen. Specific IgE and IgG antibodies were present.

Antibody titres against vegetative cells of *B. thuringiensis* were also observed in workers exposed to *B. thuringiensis* subsp. *kurstaki* (Laferrière et al., 1987, cited in WHO, 1999). Little or no formation of antibodies against spores or crystals was detected. No other adverse health effects were reported.

Doekes et al. (2004) studying more than 300 greenhouse workers, reported that exposure to *B. thuringiensis* biopesticides (Bactimos and Vectobac: subsp. *israelensis*) confers a risk of IgE-mediated sensitization. No increase in respiratory health syndromes was observed.

Two incidents of possible allergic reactions to *B. thuringiensis* have been reported to the US EPA (McClintock et al., 1995). However, neither of these was considered by the authors to be causally related to *B. thuringiensis* upon critical inspection.

New data 2016

Data provided for the first approval are considered acceptable to cover current requirements according to Regulation (EC) 1107/2009. Apart from results of a literature search, no substantial new data are submitted on sensitisation/allergenicity observations.

In the literature search covering the last 10 years and focussing toxicity or pathogenicity of Btk on mammals, two articles were identified concerning immunological effects of Btk-based MPCPs:

Hansen et al. (2010) published a study on greenhouse workers occupationally exposed to the MPCP Dipel® containing *Bacillus thuringiensis* subsp. *kurstaki* HD1 as active ingredient and to other mesophilic bacteria. HD1-like bacteria were only detected in environments where Dipel® was used. In a greenhouse with Dipel® treated tomato plants, the growers' exposure to airborne HD1-like bacteria reached 5300 CFU/m^3 and 1400 CFU/m^3 during harvest and clearing of old plants, respectively. In untreated greenhouses, the highest concentration of total mesophilic bacteria, 1.1×10^6 CFU/m^3 was detected in a cucumber greenhouse. Interestingly, the median concentrations of mesophilic bacteria in tomato greenhouses were significantly lower than in cucumber greenhouses. There was no significant difference in exposure to mesophilic bacteria in tomato greenhouses and in vegetable fields. Greenhouse workers, especially in cucumber production, are exposed to high concentrations of total bacteria during work activities. Thus, the large amount of naturally occurring airborne microorganisms is supposed to have a greater influence on the workers´ health than applied microorganisms from MPCP.

Additionally, Baelum and colleagues (2012) conducted a 3-year follow up study on sensitization and health effects of exposure to microbiological control agents used in Danish greenhouses including *Bacillus thuringiensis* subsp. *kurstaki, Verticillium lecanii*, and *Trichoderma harzianum*. For Bt 53% of the blood samples showed positive IgE levels, but no conclusion can be drawn on correlation of exposure and IgE levels as the measurement was only qualitative – a positive IgE was defined as exceeding the detection limit of 0.025 OD units. Furthermore, no difference between exposed and not exposed samples is detectable. Additionally, prevalence rate ratios among exposed increased only marginally from 1.20 (CI95%: 1.01-1.42) to 1.43 (CI95%: 1.09-1.87) over a 3-year period. Moreover, the majority of persons were exposed to more than one type of biopesticide. Despite increased IgE values to Bt which may be regarded as a sign of sensitization, no prevalence nor incidence of respiratory symptoms was detectable as there was no effect on lung function or bronchial hyperresponsiveness. Thus, the use of *B. thuringiensis* in greenhouses may give rise to sensitization while no effect on the occurrence of respiratory symptoms or lung function was observed, although the persons had a relatively long exposure.

Evaluation RMS	It has been suggested the use of *B. thuringiensis* in greenhouses may give rise to sensitization because increased IgE against *B. thuringiensis* was seen in 53% of the samples from growers over a 3-year period (Baelum et al. 2012). Despite increased IgE values to Bt which may be regarded as a sign of sensitization, neither prevalence nor incidence of respiratory symptoms was detectable. However, in another study was shown the already present airborne bacteria in greenhouses might have a greater influence on growers' health than applied biocontrol strains (Hansen et al., 2010). Overall there is no evidence in the scientific literature that bacteria including *B. thuringiensis* in greenhouses may give rise to sensitization.

Cited references:

Report: KMA 6.1.1.3/05 – Hansen, V.M., Eilenberg, J., Madsen, A.M. (2010), Occupational exposure to airborne Bacillus thuringiensis kurstaki HD1 and other bacteria in greenhouses and vegetable fields

Published report

Biocontrol Science and Technology, Vol. 20, No. 6, 2010, 605-619

Abstract:

When microorganisms are used for pest control in vegetable production, the active organisms become part of the microbiota growers are exposed to. The aim of this study was to quantify vegetable growers' exposure to the bacterial strain *Bacillus thuringiensis kurstaki* strain HD1 (termed HD1) from the biocontrol agent Dipel®, and other airborne mesophilic bacteria. Personal (n = 102) and stationary (n = 43) measurements of exposure were performed in greenhouses and open fields. Air samples were analysed by plate counts, and total counts with a microscope. Isolates resembling HD1 were identified by PCR analysis. HD1-like bacteria were only detected in environments where Dipel® was used. In a greenhouse with Dipel® treated tomato plants, the growers' exposure to airborne HD1-like bacteria reached 5300 CFU/m^3 and 1400 CFU/m^3 during harvest and clearing of old plants, respectively. In untreated greenhouses, the highest concentration of total mesophilic bacteria, 1.1×10^6 CFU/m^3, was detected in a cucumber greenhouse. The median concentrations of mesophilic bacteria in tomato greenhouses were significantly lower than the median concentrations in cucumber greenhouses. There was no significant difference in exposure to mesophilic bacteria in tomato greenhouses and in vegetable fields. We found that greenhouse workers, especially in cucumber production, were exposed to high concentrations of total bacteria. Thus, the already present airborne bacteria in greenhouses might have a greater influence on growers' health than applied biocontrol strains. However, further studies are needed to establish an occupational threshold limit for airborne bacteria and to secure a healthy working environment for vegetable growers.

Evaluation RMS	The already present airborne bacteria in greenhouses might have a greater influence on growers' health than applied biocontrol strains.

Report: KMA 6.1.1.3/06 – Baelum J., Larsen P., Doekes G., Sigsgaard T. (2012), Health effects of selected microbiological control agents. A 3-year follow-up study

Published report

Ann Agric Environ Med., 19(4):631-636

Abstract:

INTRODUCTION AND OBJECTIVES: Microbiological control agents (MBCA) are widely used in greenhouses, replacing chemical pesticides. The presented study aims to describe health effects of exposure to three types commonly used: *Bacillus thuringiensis*, *Verticillium lecanii*, and *Trichoderma harzianum* covering seven different products in greenhouse workers with emphasis on sensitization and respiratory effects.

METHODS: 579 persons aged 17 - 67 years culturing ornamental flowers were included. They were followed for three years with annual examinations including interview about exposure and symptoms, lung function, including bronchial (histamine) challenge test, and blood samples. Direct and indirect exposure for each person and year was estimated by information from respondents and employers. IgE in serum against the 7 products of MCBA was analyzed using an enzyme immunoassay technique.

RESULTS: 65%, 40%, and 78% were exposed to B. *thuringiensis*, *V. lecanii*, and *T. harzianum*, respectively, while 6, 3 and 3% were handling the products. IgE against *B. thuringiensis* was seen in 53% of the samples and with prevalence rate ratios among exposed increasing from 1.20 (CI95%: 1.01-1.42) to 1.43 (CI95%: 1.09-1.87) over the 3-year period. There was no relation between exposure to any MBCA and neither prevalence nor inci-

dence of respiratory symptoms and there was no effect on lung function or bronchial responsiveness.

CONCLUSIONS: Use of *B. thuringiensis* in greenhouses may give rise to sensitization while no effect on the occurrence of respiratory symptoms or lung function was observed. The persons had a relatively long exposure. Therefore, a healthy worker effect may have influenced the results.

Evaluation RMS	Increased IgE against *B. thuringiensis* was seen in 53% of the samples from growers over a 3-year period (Baelum et al. 2012). Despite increased IgE values to Bt which may be regarded as a sign of sensitization, neither prevalence nor incidence of respiratory symptoms was detectable. Therefore, it cannot be concluded that use of *B. thuringiensis* in greenhouses may give rise to sensitization.

B.6.1.1.4 Direct observation, e.g. clinical cases

Pearce et al. (2002a) carried out a study on aerial spraying of an urban area and studied 58 children with asthma living inside and outside of the spray zone. There was no evidence of adverse effects from the use of bioinsecticide Foray 48B (*B. thuringiensis* subsp. *kurstaki* HD-1). Low-level exposure did not influence asthma symptoms in predisposed children.

Another cohort study was conducted following the effect of aerial spraying with Foray 48B in New Zealand (Petrie et al., 2003). Residents inside spray zone were tested before and after spraying. A doubling in the rate of irritated throat symptoms was noted. Gastrointestinal symptoms increased significantly (stomach and gas discomfort, diarrhoea). Hay fever sufferers had increased symptoms following spraying (hay fever season in this period). This study suggests that aerial spraying with Foray 48B is associated with some adverse health effects (upper airway and gastrointestinal symptoms). However, since only 62% of individuals enrolled in the pre-application survey responded to the post-application survey, over-reporting by affected residents may confound the data. Furthermore, no control group (residents living outside the spray zone) were included in the study.

In another study a randomly selected population (1009 individuals) was interviewed pre and post spraying with Foray 48B. No measurable health effects could be detected (Pearce et al., 2002b).

An epidemiological study in Oregon during two seasons of aerial spraying with *B. thuringiensis* subsp. *kurstaki* of more than 80,000 residents resulted in 55 *B. thuringiensis* positive cultures. Fifty-two of the isolates were assessed to be contaminants and not the cause of human illness, and only for three patients, *B. thuringiensis* could neither be ruled in nor out as the pathogen (Green et al., 1990).

New data 2016

Data provided for first approval are considered acceptable to cover current requirements. Neither new studies nor substantial new information is submitted for renewal of Btk SA-12 according to Regulation (EC) No 1107/2009. No additional information on clinical cases of Btk is reported in open peer-reviewed literature (please refer to the literature research report Seehase 2016, KMA 6.1.1/07).

No harmful effects have been observed on populations exposed to Btk-based products.

Evaluation RMS	There are no major effects observed on populations exposed to aerial spraying of bioinsecticides based on *B. thuringiensis* subsp. *kurstaki*. In the few reports indicating an effect it is not clear that the symptoms observed are due to exposure to *B. thuringiensis*-based insecticides.

B.6.1.2 Basic studies

B.6.1.2.1 Sensitisation

No data have been provided for first approval of Btk SA-12 since the available methods for testing dermal sensitisation are not suitable for testing microorganism and no methods exist for testing sensitisation by inhalation.

New data 2016

According to Regulation (EC) 283/2013 (footnote 1 to point 5.2.1 in Part B), the available methods for testing dermal sensitisation are not suitable for testing microorganisms as microorganisms do not penetrate the skin. Therefore, no new studies are submitted for renewal of the approval of Btk SA-12 under Regulation (EC) No 1107/2009.

In the literature search covering the last 10 years and focussing on toxicity, pathogenicity, or sensitisation of Btk on mammals, one article was identified showing increased IgE levels to Btk (Baelum et al, 2012). For more information, please refer to point B.6.1.1.3 above.

Evaluation RMS	No data have been provided for first approval or renewal of Btk SA-12 since the available methods for testing dermal sensitisation are not suitable for testing micro-organism and not relevant as microorganisms do not penetrate the skin. No methods exist for testing sensitization by inhalation and there is no evidence in the scientific literature that bacteria including *B. thuringiensis* may give rise to sensitization.

B.6.1.2.2 Acute toxicity, pathogenicity and infectiveness

Acute oral toxicity, pathogenicity and infectiveness

Report:	**KMA 6.1.2.2/01** [redacted] (1992) ACUTE ORAL TOXICITY STUDY OF SA12 IN RATS
Test substance/concentration:	*Bacillus thuringiensis* subsp. *kurstaki* strain SA-12, Lot no. EC1614-145/given by sponsor: 1 x 10^8 CFU/ml; determined by laboratory: 9.6 x 10^8 CFU/ml
Guideline/GLP:	Corresponding to US EPA OPPTS 885.3050/Yes
Deviations:	i) only 8-day observation period (according to guideline 21 days), ii) no untreated control animals included in the study, iii) no infectivity/persistence and clearance data and iv) necropsy was not performed.
Acceptability:	**Yes**
Species/strain:	Rat/Sprague-Dawley
Doses/no. of animals:	Single dose of 1.2 x 10^8 CFU per rat/3 rats per sex
Administration way/vehicle:	Oral by gavage/phosphate-buffered saline
Test system:	All animals were observed 1 and 4 hours following treatment, and once daily thereafter for an 8-day period for mortality and clinical signs of toxicity. Body weights were recorded on day of dosing and Day 8.

Findings:

Mortality: None

Clinical signs: No treatment-related effects

Body weight: Not affected

Infectivity/clearance: Not determined

Necropsy: Not performed

Conclusion:

No adverse effects were observed. However the study gives no information on infectivity/persistence and clearance from gut and other organs.

RMS comment:

Since the animals have only been observed throughout an 8-day period, instead of the 21-day period requested by the guideline, the study could not be used to set an LD_{50}.

Because of the nature of the bacteria it is believed that the organism would have a slow clearance from the gastrointestinal tract and also translocation to other organs has been observed with *B. thuringiensis* species.

Report:	**KMA 6.1.2.2/02** [redacted] (1999A) COSTAR TECHNICAL CONCENTRATE. ACUTE ORAL TOXICITY STUDY IN RATS
Test substance/concentration:	*Bacillus thuringiensis* subsp. *kurstaki* strain SA-12, Lot no. 2731269/
Guideline/GLP:	US EPA OPPTS 870.1100/Yes
Deviations:	None. However, the guideline used is for chemical pesticides.
Acceptability:	**Yes**
Species/strain:	Rat/Sprague-Dawley
Doses/no. of animals:	Single dose of 5050 mg/kg b.w./5 rats/sex
Administration way/vehicle:	Orally by gavage/deionized water
Test system:	Animals were observed for mortality and signs of toxicity three times on the day of dosing and once daily thereafter for 14 days. Body weights were recorded prior to dosing and on days 7 and 14.

Findings:

Mortality: None

Clinical signs: Oily anal discharge in all males, diarrhoea in 1/5 male and 1/5 female on the day of treatment. Decreased defecation in one male was observed the day after treatment. Animals were asymptomatic by Day 2.

Body weight: Not affected

Infectivity/clearance: Not studied

Necropsy: Discoloured liver in 3/5 males and discoloured kidney in one other male

Conclusion:

LD_{50} > 5050 mg/kg bw. Transient gastrointestinal symptoms. Discolouration of liver and kidney was found in 4 out of 5 male rats. Furthermore, no conclusion can be drawn on infectivity/persistence and clearance, since no data are presented.

RMS comment:

The study is missing data on infectivity/persistence and clearance as required by Directive 2001/36/EEC. The gastrointestinal symptoms observed in the beginning of the study are transient, but may show the potential of the strain to cause diarrhoea also in humans.

There are no explanations for the discolouration of liver and kidney observed in 4 out of 5 males, and since no control group is included in the study and no data are given on the microbial counts in the organs, it is difficult to assess the importance of the observation. Besides the discolouration, no toxic effects are observed in the organs.

These two studies lack, as indicated, data on infectivity/persistence and clearance from the gastrointestinal tract and other organs/tissue. However, this has been studied in the 13-week oral dosing study with the strain SA-11 ([redacted] 1993) (B.6.1.2.5.1), a study that can be considered as a worst-case study, and therefore no further oral studies are requested. In the 13-week study, no translocation to other organs was observed. The strain was detected at high level in caecum at termination of the study in all rats, but was cleared from this site after the 4-week recovery period. Furthermore the strain was detected in the lung of some animals both at dosing termination and after the recovery period, presumably caused by aspiration of the strain during dosing.

Evaluation RMS	The study by [redacted], 1992 on Btk strain SA-12 performed according to OPPTS 870.3050 revealed no toxicity upon acute oral exposure. Since the animals have

	only been observed throughout an 8-day period, instead of the 21-day period requested by the guideline, the study could not be used to set an LD_{50}. Another study by [redacted], 1999a conducted according to the guideline US EPA OPPTS 870.1100 for chemical pesticides showed no toxicity upon acute oral exposure (LD_{50} > 5050 mg/kg bw). However, the transient discolouration of liver and kidney observed in 4 out of 5 males could not be explained. Both studies lack data on infectivity/persistence and clearance from the gastrointestinal tract and other organs/tissue. At the Pesticides Peer Review meeting M4 it was decided that EFSA should indicate in the conclusion that the applicant has not addressed persistence and clearance in the acute oral rat toxicity study. However, it was agreed more information on this was not required to complete the EU level risk assessment.

New data 2016

For renewal of Btk SA-12, a new study conducted according to OPPTS 885.3050 Microbial pesticide test guidelines with the product Thuricide SC, a liquid formulation of Btk SA-12 is submitted (10^8 CFU/animal administered oral, observation for 21 days, clearance determination,). The study is therefore considered applicable for the evaluation of possible toxicity, pathogenicity and infectivity of Btk SA-12. No mortality and no signs of toxicity or pathogenicity have been observed. Bacterial load was cleared within 7 days.

In the literature search covering the last 10 years and focussing on toxicity or pathogenicity of Btk to humans and mammals, two articles were identified. A study by Wilcks et al. (2006a) on the effect of feeding *Bacillus thuringiensis* (subsp. *israelensis* and subsp. *kurstaki*) to human-flora associated rats revealed no evidence of *B. thuringiensis* subsp. *kurstaki* causing acute oral toxicity in mammals. Another study by Berlitz and colleagues (2012) investigated the gastro-intestinal tract and stomach content of mice after oral application of 2×10^9 CFU Bt 1958-2, Bt 2014-2, and the BTh Thuricide 63 and did also not detect any toxic effects.

Thus, there is no evidence that Btk may cause acute oral toxicity, pathogenicity or infectivity in mammals.

Reference:	**KMA 6.1.2.2/03**
Report:	[redacted] (2015a) Thuricide SC - Acute oral toxicity/Pathogenicity study in rats (*Rattus norvegicus*) Unpublished Report No:RL1372/2015PO-B
Guideline(s):	OPPTS 885.3050. Microbial pesticide test guidelines. Acute oral toxicity/pathogenicity (1996)
Deviations:	Maximum mean humidity registered (74.4%) was higher than range (30 - 70%) Evaluation of the 10th of shelf control animal was not carried out These deviations did not affect the study.
GLP:	Yes
Acceptability:	Acceptable
Duplication: **(if vertebrate study)**	Yes

Executive summary

An acute oral toxicity study was conducted according to OPPTS 885.3050 Microbial pesticide test guidelines. Four groups of 3 female and 3 male Wistar rats fasted overnight were dosed with 0.1 ml of the undiluted test item Thuricide SC (containing the active ingredient Btk SA-12) as single gavage, ensuring the administration of 5.4×10^8 CFU animal. Additionally, one negative and one witness control group were included (2 animals/sex each). The experimental groups were observed 3, 7, 14, and 21 days post application. Animals were sacrificed and blood, organs (brain, lymph node, kidney, lung, liver, spleen) and faeces were analysed for MCA. Control groups were observed on day 21.

No toxic signs, pathogenicity, or mortality occurred. No pathological changes were observed at necropsy. MCA was only detected in faeces of animals after 6 hours and 3 days, but not on day 7, 14, and 21 as well as the control animals.

Thuricide SC containing the active ingredient Btk SA-12 is of low toxicity and will not require classification. Bacterial load was cleared within 7 days.

Material and Methods

Test Item

Designation	Thuricide SC
Characteristics	Suspension concentrate
Batch no.	001-14-14400
Expiration date	August 2016
Purity	5.4×10^9 CFU/mL of Btk SA-12

Test System

Species	*Rattus norvegicus*, Wistar (albino rats)
Age	8 - 10 weeks
Body weight	Males: 261 – 278 g Females: 150 – 184 g
Source	[redacted]
Number	32 animals, 3 males and 3 females per each experimental group; 2 males and 2 females per each control group)
Acclimatisation period	6 days

Test Conditions

Housing	2 - 3 rats per cage
Food	Pelleted commercial diet for the species (Nuvilab CR1, Quimtia S.A.) and tap water, both provided *ad libitum*
Temperature	21.8 – 23.7°C
Photoperiod	12 h artificial light and 12 h darkness
Humidity	62.3 – 74.4%

Study Design and Methods

In-life dates	10 – 31.07.2015
Exposure	0.1 mL of 5.4×10^9 CFU/mL resulting in 5.4×10^8 CFU per animal
Vehicle	None, undiluted
Post exposure observation:	21 days
Experimental treatment	The test item used was Thuricide SC containing Btk SA-12 as active substance. Four groups 3 male and 3 female Wistar rats fasted overnight received a single dose of 5.4×10^8 CFU/mL Thuricide SC (undiluted) as single gavage. Two control groups
Observations	Animals were observed for signs of toxicity and mortality 3, 7, 14, and 21 days post-dosing. Additionally, faeces of all animals were collected and evaluated 6 h post dosing as well as on days of necropsy to quantify MCA and thus assess the elimination rate. The rats were observed daily for clinical signs of toxicity/pathogenicity. Gross pathological examination was perform on terminally sacrificed animals. MCA quantification was performed on

blood, faeces, and organs (brain, lung, lymph nodes, kidney, spleen, liver; pooled).

Findings

No mortality occurred. No clinical signs of toxicity or pathogenicity were observed after single exposure to 5.4 × 10^8 CFU per animal. Only normal gain in body weight was observed. Gross pathological examination (external and visceral) did not reveal any lesion of pathological significance in terminally sacrificed rats. MCA quantification revealed recoveries of > 3.0 ×10^3 CFU/mL in three stool samples 6 hours and 3 days post application. No recovery was observed in the samples of 7, 14, and 21 days post application as well as in any of the control groups.

Conclusion

As no mortality or signs of toxicity or pathogenicity were observed in the rats after application of 5.4 × 10^8 CFU/animal, it is concluded, that Btk SA-12 does not warrant classification as being toxic or harmful based on its acute oral toxicity. The estimated clearance is 7 days.

RMS evaluation	For renewal of Btk SA-12, a new study conducted according to OPPTS 885.3050 Microbial pesticide test guidelines with a liquid formulation of Btk SA-12 is considered applicable for the evaluation of possible toxicity, pathogenicity and infectivity of Btk SA-12 ([redacted]2015). As no mortality or signs of toxicity or pathogenicity were observed in the rats after application of 5.4 × 10^8 CFU/ animal, it is concluded, that Btk SA-12 does not warrant classification as being toxic or harmful based on its acute oral toxicity. The estimated clearance is 7 days.
Endpoint: Acute oral infectivity, toxicity and pathogenicity:	No signs of toxicity, pathogenicity or infectivity have been detected upon single oral exposure to Btk SA-12 or a liquid formulation of Btk SA-12. $LD_{50\ rat}$ > 5.4 × 10^8 CFU/animal

Cited references:

Reference	**KMA 6.1.2.2/04**
Report	Wilcks, A., Hansen, B.M., Hendriksen, N.B., Licht, T.R. (2006a) Persistence of *Bacillus thuringiensis* bioinsecticides in the gut of human-flora-associated rats Published report FEMS Immunol Med Microbiol, 48, 2006, pp 410-418
Guideline	Not applicable
GLP	No

Abstract

The capability of two bioinsecticide strains of *Bacillus thuringiensis* (ssp. *israelensis* and ssp. *kurstaki*) to germinate and persist *in vivo* in the gastrointestinal tract of human-flora-associated rats was studied. Rats were dosed either with vegetative cells or spores of the bacteria for 4 consecutive days. In animals fed spores, *B. thuringiensis* cells were detected in faecal and intestinal samples of all animals, whereas vegetative cells only poorly survived the gastric passage. Heat-treatment of intestinal samples, which kills vegetative cells, revealed that *B. thuringiensis* spores were capable of germination in the gastrointestinal tract. In one animal fed spores of *B. thuringiensis* ssp. *kurstaki,* these bacteria were detected at high density (10^3 - 10^4 CFU/ g faecal and intestinal samples) even 2 weeks after the last dosage. In the same animal, passage of *B. thuringiensis* ssp. *kurstaki* to the spleen was observed; however, no other adverse effects were observed. Denaturing gradient gel electrophoresis of PCR-amplified bacterial 16S rRNA genes in faecal samples revealed no major effect of *B. thuringiensis* on the composition of the indigenous gut bacteria. Additionally, no cytotoxic effect was detectable in gut samples by Vero cell assay.

Materials and Methods

Groups of six germfree Sprague-Dawley rats (7-9 weeks old) were used to produce human-flora-associated (HFA) rats (Wilcks et al. 2006b) and dosed for 4-consecutive days with *B. thuringiensis* strains either (1) irradiated spores (control), (2) untreated spores, (3) heat-treated spores, or (4) vegetative cells.

Rats fed *B. thuringiensis* ssp. *kurstaki* DMU67R (Btk) received either 10^7 spores (untreated or heat-treated) or $10^7 – 10^8$ vegetative cells per day for 4-consecutive days. Animals dosed with *B. thuringiensis* ssp. *israelensis* HD567 (Bti) received 10^8 untreated spores, 10^6 heat-treated spores, or 10^8 vegetative cells.

Half of the animals were sacrificed one day post dosing (day 5), and the remaining half at 14 days post dosing (day 18).

Findings

B. thuringiensis cells were detected in faecal and intestinal samples (duodenum, ileum, caecum, colon) of all animals, whereas vegetative cells only poorly survived the gastric passage. No difference between Btk recovered from faecal samples of rats dosed with untreated and those dosed with heat-treated spores was detectable. In 5/6 animals, Btk was detectable 14 days post administration in the faeces. In one animal spores of Btk were detected at high density ($10^3 – 10^4$ CFU/g faecal and intestinal samples) even 14 days after the last dosage. In the same animal, passage of Btk to the spleen was observed; however, no other adverse effects were detected. Denaturing gradient gel electrophoresis of PCR-amplified bacterial 16S rRNA genes in faecal samples revealed no major effect of *B. thuringiensis* on the composition of the indigenous gut bacteria. Additionally, no cytotoxic effect was detectable in gut samples by Vero cell assay revealing no enterotoxin production.

Conclusion

Heat-treatment of *B. thuringiensis* spores prior to dosing did not affect their activity in the gastrointestinal tract of HFA rats, however, no health issues associated with *Btk* or *Bti* have been revealed, except one single animal showing *Btk* in the spleen.

Evaluation RMS	The study is applicable and acceptable. The effect of feeding *Bacillus thuringiensis* (subsp. *isralensis* and subsp. *kurstaki*) to human-flora associated rats revealed no evidence of *B. thuringiensis* subsp. *kurstaki* causing acute oral toxicity in mammals.

Reference	**KMA 6.1.2.2./05**
Report	Berlitz, D.S., Giovenardi, M., Charles, J.F., Fiuza, L.M. Toxicity intraperitoneal and intragastric route *of Bacillus thuringiensis* and *Melia azedarach* in mice Puplished report Arq. Inst. Biol., São Paulo, 79 (4), pp. 511-517, 2012
Guideline:	Not applicable
GLP:	No

Abstract:

The aim of this investigation was the assessment of toxicity of two new isolates of *Bacillus thuringiensis*, and the aqueous extract of *Melia azedarach* through in vivo assays in CF1 mice. Bt 1958-2, Bt 2014-2 and the BTh Thuricide 63 standard isolates were grown in liquid usual glicosed medium, and Cry proteins were purified by centrifugation on a sucrose gradient. The supernatant was autoclaved at 121ºC, 15 min. to maintain the exotoxins. Dehydrated leaves of *M. azedarach* were used to prepare a 10% aqueous extract. Mice were treated either orally or intraperitoneally with a whole bacterial suspension (1×10^{10} UFC/mL), a culture supernatant or purified crystal protein (50 µg/mL), and with the plant extract (50 µg/mL). The stomachs of the mice were collected and observed in stereomicroscopy, and the stomach contents were analyzed in 10% SDS-PAGE. Results showed that none of the oral treatments were toxic to mice, but intraperitoneal bacterial suspensions were lethal to the animals 6 - 24 hours after injection. In conclusion, the Cry proteins of the new *B. thuringiensis* isolates must be evaluated for their use as tools in the biotechnology field, since they do not show toxicity against mammals, intragastrically or peritoneally, just like the *M. azedarach* aqueous extract (10%), with those being indicated for the biological control of pest insects.

Material & Methods

12 groups of 5 adult, male mice (CF1 strain), aged 80 - 100 days, were orally treated with 200 µl test item, either 50 µg cry proteins or 1×10^{10} CFU/mL from Bt 1958-2, Bt 2014-2, or BTh Thuricide 63 isolates (= 2×10^9 CFU/animal). Suspension, supernatant and pellet were prepared from Bts grown in UG liquid medium (30°C, 180 rpm). Purification of cry-Proteins present in the pellet was performed via ultracentrifugation on sucrose gradient. Treatments were carried out through gavage for 0, 12, and 24 h, at cumulative doses. Total amount of faeces was carried out 24 and 48 h post application. Animals were sacrificed at 48 h post application. Their stomach content was analysed in a 10% SDS-Page and their stomachs were analysed via stereomicroscopy.

Findings

No adverse symptoms were observed in mice after oral application of 2×10^9 CFU Bt. The protein profile of the stomach content and faeces from mice treated with Bt, assessed in 10% SDS-PAGE, reveal bands of different sizes compared to control. Microscopic examination of the stomachs of treated mice did not show any damage compared to control. No treatment related differences could be detected in evaluations of SDS-PAGE of faeces of orally treated mice.

Conclusion:

The protein profile data of the stomach content and faeces suggest that *B. thuringiensis* proteins are degraded by the mammals' digestive system. Oral treatment of bacterial suspension and cry proteins did not show any toxic effects.

Evaluation RMS	Mice were treated either orally or intraperitoneally with a whole bacterial suspension (1.10^{10} UFC/mL from Bt 1958-2, Bt 2014-2, or BTh Thuricide 63 isolates (= 2×10^9 CFU/animal)), a culture supernatant or purified crystal protein (50 µg/mL), and with the plant extract (50 µg/mL). No adverse symptoms were observed in mice after oral application of 2×10^9 CFU Bt. However, intraperitoneal bacterial suspensions were lethal to the animals 6 - 24 hours after injection. Application of cry-proteins and supernatants revealed no mortality 72 h post application. The RMS have asked the applicant if BTh Thuricide 63 contains Btk SA-12. The applicant has stated that BTh Thuricide 63 referenced in the study is not a culture owned or recognized by Certis USA LLC and does not contain Btk SA-12. Certis USA LLC is the owner of the product and trademark Thuricide® containing Btk SA-12. Furthermore it appears from the article that the Bt strains in BTh Thuricide 63 may belong to serovar *thuringiensis* 1, a known beta-exotoxin producer. Consequently, the results of this study are considered not relevant for the risk assessment of approved Btk strains, which don't produce β-exotoxins.

Acute inhalation toxicity, pathogenicity and infectiveness

No strain specific data have been provided for first approval of SA-12.

New data 2016

For renewal of Btk SA-12, an acute inhalation toxicity study conducted according to OPPTS 885.3150 Microbial pesticide test guidelines in rats with the product Thuricide SC is submitted meeting the requirements for microbial pathogenicity testing (10^8 CFU/animal administered intranasal, observation for 21 days, clearance determination). The study is therefore considered applicable for the evaluation of possible toxicity, pathogenicity, and infectivity of Btk SA-12. Under the conditions of the study, the test item did not reveal mortality or adverse effects after acute respiratory exposure.

Additionally, in the literature search covering the last 10 years and focussing on toxicity of Btk on mammals, two articles were identified studying respiratory toxicity of mice following exposure to Btk (Barfod et al., 2010, Tayabali et al., 2011). Both published studies demonstrate that Btk does not produce marked effects in mice.

Thus, there is no evidence that Btk SA-12 may cause acute respiratory toxicity, pathogenicity or infectivity in mammals.

Reference:	**KMA 6.1.2.2/07**
Report:	[redacted] (2015b) Acute pulmonary toxicity/pathogenicity study in rats (*Rattus norvegicus*) Unpublished Report No: RL1398/2015PP-B
Guideline(s):	OPPTS 885.3150. Microbial pesticide test guidelines. Acute pulmonary toxicity/ pathogenicity (1996)
Deviations:	Maximum mean humidity registered (74.4%) was higher than range (30 - 70%) This deviation did not affect the study.
GLP:	Yes
Acceptability:	Acceptable
Duplication: **(if vertebrate study)**	No

Executive summary

An acute pulmonary toxicity study was conducted according to OPPTS 885.3150 Microbial pesticide test guidelines. Five groups of 3 female and 3 male Wistar rats received 0.05 mL of the test item Thuricide SC (containing the active ingredient Btk SA-12) diluted in demineralized water at a final concentration of 5.4×10^9 CFU/mL (1.35×10^8 CFU/animal) intranasally. Additionally, one negative and one witness control group were included (2 animals/sex). The experimental groups were sacrificed 1 h, and 3, 7, 14, and 21 days post application. Blood, organs (brain, lymph node, kidney, lung, liver, spleen) and caecum content were analysed for MCA. Control groups were observed on day 21.

No obvious clinical signs of toxicity or pathogenicity occurred in any group. No pathological changes were observed at necropsy. MCA was only detected in lungs of animals analysed 1 hour and 3 days post application, but not on day 7, 14, and 21 as well as the control animals.

Thuricide SC containing the active ingredient Btk SA-12 is of low toxicity based on the NOAEL at 1.35×10^8 CFU/mL and will not require classification. The estimated clearance is 7 days.

Material and Methods

Test Item

Designation	Thuricide SC
Characteristics	Suspension concentrate,
Batch no.	001-14-14400
Expiration date	August 2016
Purity	5.4×10^9 CFU/mL of Btk SA-12

Test System

Species	*Rattus norvegicus*, Wistar (albino rats)
Age	8 - 11 weeks
Source	[redacted]
Number	38 animals, 3 males and 3 females per each experimental group; 2 males and 2 females per each control group
Acclimatisation period	6 days

Test Conditions

Housing	2 - 3 rats per cage

Food	Pelleted commercial diet for the species (Nuvilab CR1, Quimtia S.A.) and tap water, both provided *ad libitum*
Temperature	21.8 – 23.7°C
Photoperiod	12 h artificial light and 12 h darkness
Humidity	60.3 – 74.4%

Study Design and Methods

In-life dates	10. – 31.03.2015
Exposure	Intranasal
Vehicle	Demineralized water
Post exposure observation:	21 days
Experimental treatment	The test item used was Thuricide SC containing Btk SA-12 as active substance. Five groups 3 male and 3 female Wistar rats received a single dose of 0.05 mL of the test item diluted in demineralized water at a final concentration of 2.7×10^9 CFU/mL (1.35×10^8 CFU/animal) intranasally.
Observations	Animals were observed for signs of toxicity and mortality 1 h, and 3, 7, 14, and 21 days post-dosing. The rats were observed daily for clinical signs of toxicity/pathogenicity. Gross pathological examination was performed on terminally sacrificed animals. MCA quantification was performed in lung, blood, further organs (brain, lymph nodes, kidney, spleen, liver; pooled) and caecum content.

Findings

No mortality occurred. No clinical signs of toxicity or pathogenicity were observed after single intranasal exposure to 1.35×10^8 CFU/animal. Only normal gain in body weight was observed. Gross pathological examination (external and visceral) did not reveal any lesion of pathological significance in terminally sacrificed rats. MCA quantification revealed recoveries of $> 3.0 \times 10^3$ CFU/mL in lung samples 1 hour and 3 days post application. No recovery was observed in the samples of 7, 14, and 21 days post application as well as in any of the control groups.

Conclusion

As no mortality or signs of toxicity or pathogenicity were observed in the rats after intranasal application of 1.35×10^8 CFU/animal, it is concluded, that Btk SA-12 does not warrant classification as being toxic or harmful based on its acute pulmonary toxicity. The estimated clearance is 7 days.

RMS evaluation	For renewal of Btk SA-12, an acute inhalation toxicity study (conducted according to OPPTS 885.3150 showed no mortality or signs of toxicity or pathogenicity in rats after intranasal application of 1.35×10^8 CFU/animal. The study meets the requirements for microbial pathogenicity testing and is therefore considered applicable. It is concluded, that Btk SA-12 does not warrant classification as being toxic or harmful based on its acute pulmonary toxicity. The estimated clearance is 7 days.
Endpoint: Acute intratrachal/ inhalation infectivity, toxicity and pathogenicity:	No toxicity, infectivity and pathogenicity upon pulmonary exposure observed $LC_{50} > 1.35 \times 10^8$ CFU Btk SA-12/animal

Cited references:

Reference	**KMA 6.1.2.2/08**
Report	Barfod, K.K., Poulsen, S.S., Hammer, M., Larsen, S.T. (2010)
	Sub-chronic lung inflammation after airway exposures to *Bacillus thuringiensis* biopesticides in mice.
	Published report
	BMC Microbiol 2010, 3, 10:233
Guideline:	Not applicable
GLP:	No

Abstract

BACKGROUND: The aim of the present study was to assess possible health effects of airway exposures to *Bacillus thuringiensis* (*Bt*) based biopesticides in mice. Endpoints were lung inflammation evaluated by presence of inflammatory cells in bronchoalveolar lavage fluid (BALF), clearance of bacteria from the lung lumen and histological alterations of the lungs. Hazard identifications of the biopesticides were carried out using intratracheal (i.t.) instillation, followed by an inhalation study. The two commercial biopesticides used were based on the *Bt*. subspecies *kurstaki* and *israelensis*, respectively. Groups of BALB/c mice were i.t instilled with one bolus (3.5×10^5 or 3.4×10^6 colony forming units (CFU) per mouse) of either biopesticide. Control mice were instilled with sterile water. BALFs were collected and the inflammatory cells were counted and differentiated. The BALFs were also subjected to CFU counts.

RESULTS: BALF cytology showed an acute inflammatory response dominated by neutrophils 24 hours after instillation of biopesticide. Four days after instillation, the neutrophil number was normalised and inflammation was dominated by lymphocytes and eosinophils, whereas 70 days after instillation, the inflammation was interstitially located with few inflammatory cells present in the lung lumen. Half of the instilled mice had remaining CFU recovered from BALF 70 days after exposure. To gain further knowledge with relevance for risk assessment, mice were exposed to aerosols of biopesticide one hour per day for 2×5 days. Each mouse received 1.9×10^4 CFU Bt israelensis or 2.3×10^3 CFU *Bt kurstaki* per exposure. Seventy days after end of the aerosol exposures, 3 out of 17 mice had interstitial lung inflammation. CFU could be recovered from 1 out of 10 mice 70 days after exposure to aerosolised *Bt kurstaki*. Plethysmography showed that inhalation of Bt aerosol did not induce airway irritation.

CONCLUSIONS: Repeated low dose aerosol exposures to commercial Bt based biopesticides can induce subchronic lung inflammation in mice, which may be the first step in the development of chronic lung diseases. Inhalation of Bt aerosols does not induce airway irritation, which could explain why workers may be less inclined to use a filter mask during the application process, and are thereby less protected from exposure to Bt spores

Material and Methods

Bacterial suspensions were prepared from the commercially available insecticides Vectobac® (*Bt israelensis*) and Dipel® (*Bt kurstaki*), both from Valent Biosciences (Sumitomo Chemical Agro Europe, Lyon, France).

Groups of ten BALB/c mice (Taconic M&B, Ry, Denmark) were i.t instilled with one bolus (3.5×10^5 CFU Btk or 3.4×10^6 CFU Bti per mouse) of either biopesticide or sterile water as control. After 4 hours, 24 h, 4 days, and 70 days mice were sacrificed, bronchoalveolar lavage fluid (BALF) was collected, and CFU and inflammatory cells were assessed. For each mouse, 200 cells were counted and differentiated. Values are expressed as means with SEM. Histology was performed 70 days after exposure.

Findings

A significant neutrophilic influx was seen 24 hours post exposure for both biopesticides. Four days after instillation, the neutrophil number was normalised and macrophages represented the predominant cell type in BALF. 70 days after instillation, the inflammation was interstitially located with few inflammatory cells present in the lung lumen. Bacteria in CFU counts of BALF were still present 70 days post exposure in 8 of 10 mice treated with Vectobac® (3.4×10^6 CFU Bti) and 1 out of 9 mice treated with Dipel® (3.5×10^5 CFU Btk) with an average of 150 and 2850 CFU/BALF, respectively.

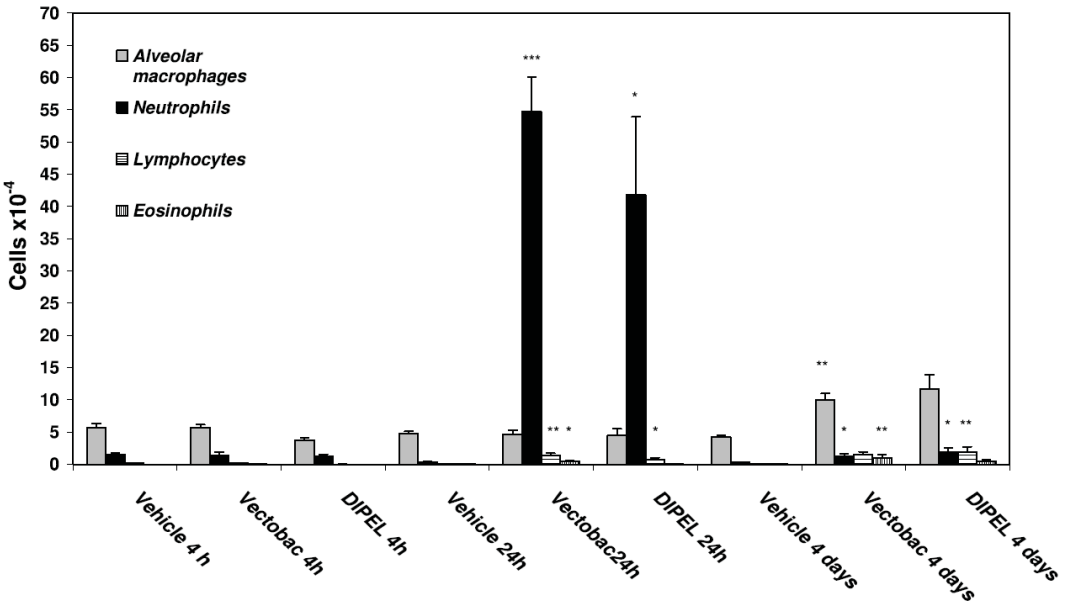

Fig. 6.1.2.2-1: Cells in bronchoalveolar lavage fluid at different time points after instillation of biopesticides

Conclusion

Acute exposure to Bt based biopesticides induced an influx of neutrophilic granulocytes in BALF, which was reversible after 4 days and represents a typical inflammatory response to an extern stimulus. 70 days post exposure, slight tissue changes as a sign of interstitial inflammation were observed in both groups, however, histological pictures of lung tissue from control animals are only shown in low magnification, compared to treated animals, and do not allow a conclusive evaluation of the effect.

Evaluation RMS	Agree with the conclusion

Reference	**KMA 6.1.2.2/09**
Report	Tayabali, A.F., Nguyen, K.C., Seligy, V.L. (2011) Early murine immune responses from endotracheal exposures to biotechnology-related *Bacillus* strains Published report Toxicological & Environmental Chemistry, 2011, Vol. 93, pp. 314-331
Guideline:	Not applicable
GLP:	No

Abstract

An immunology-based *in vivo* screening regime was used to assess the potential pathogenicity of biotechnology-related microbes. Strains of *Bacillus cereus* (Bc), *Bacillus subtilis* (Bs), *Bacillus thuringiensis* (Bt), and Bt commercial products (CPs) were tested. Balb/c mice were endotracheally instilled with purified spores, diluted CP, or vegetative cells (VC) (live or dead). Exposed mice were evaluated for changes in behavioural and physical symptoms, bacterial clearance, pulmonary granulocytes, and pulmonary and circulatory pyrogenic cytokines (interleukins (IL)-1β, IL-6 and tumour necrosis factor (TNF)-α), as well as acute phase biomarkers (fibrinogen and serum amyloid A). Except for some differences in clearance rates, no marked effects were observed in mice exposed to any spore at 10^6 or 10^7 colony forming units (cfu). In contrast, live Bc or Bt VCs (10^5 or 10^6 cfu) produced shock-like symptoms (lethargy, hunched appearance, ruffled fur, and respiratory distress), and 11-200-fold elevations in pyrogenic cytokines at 2-h post-exposure. In the study, 4-h effects included increased lethargy, ocular discharge, and 1.5-4-fold rise in circulatory acute phase markers, but no indications of recovery. Bs VC did not produce any changes in symptoms or biomarkers. After 2 or 4 h of exposure to dead VC, increases of only plasma IL-1beta and TNF-α (4.6- and 12.4-fold, respectively) were observed. These findings demonstrate that purified spores produced no marked effects in mice compared to that of metabolically active bacteria. This

early screening regime was successful in distinguishing the pathogenicity of the different *Bacillus* species, and might be useful for assessing the relative hazard potential of other biotechnology-related candidate strains.

Material and Methods

Live and dead vegetative cells were prepared from spores of the ATCC strains: *B. cereus* (Bc14579TM), *B. subtilis* (Bs6051aTM), *B. thuringiensis* (Bt13367TM), and the commercial products: Foray 48B, *Bt* subsp. *kurstaki* (= CP1) and Vectobac 12AS, *Bt* subsp. *israelensis* (= CP2).

Endotracheal instillation was performed in anesthetized (isoflurane) female Balb/c mice (aged 8 - 10 weeks) using a 25 µL dose of bacteria or saline alone aerosolized through a microsprayer.

Animals were sacrificed 2h post exposure with 10^6 cfu per mouse (pilot experiment revealed this time point). Blood was collected by cardiac puncture, blood plasma was stored at -80°C for further analysis of circulating, pyrogenic cytokines, as well as Th1 and Th2 cytokines. Additionally, tissues (lung, liver, trachea) were collected and also stored at -80°C. In order to assess clearance of the vegetative cells from tissue, animals were sacrificed at different time points, tissues were excised and homogenized prior to cfu determination.

Granulocyte infiltration was monitored by immunofluorescence microscopy.

Findings

Data revealed that 99% of pulmonary clearance occurred between 20 and 120 min post exposure. Bs was cleared within 48 h, at least 2 days before the other bacteria. The commercial Bt preparations showed delayed clearance from both the trachea and lungs since bacteria could still be recovered 3 – 7 days post-exposure. Mice exposed to Bt ssp. israelensis showed an apparent transitory fall in pulmonary and tracheal bacteria at 24 h post-exposure, followed by an elevation in bacterial counts in both tissues. Mice treated with 10^6 purified spores or commercial product showed no apparent symptoms over a 1-week period. Mice treated with 10^7 spores had noticeable ruffled fur at 24 h, but otherwise resembled saline-treated controls. Experiments involving washed Bc and Bt VC were prematurely terminated at 2 h due to animal welfare aspects. Purified spores and diluted commercial products revealed no significant changes on cytokine level compared to control when monitored over a 1-week period. However, compared to control, washed Bc, Bt, and Btk vegetative cells showed a significant increase in granulocytes, but not on cytokine level. Only lung tissue of Bc treated animals showed a significant increase in pro-inflammatory cytokines compared to controls.

Conclusion

Spores at concentrations up to 10^7 CFU per mouse and live vegetative cells up to 10^4 CFU per mouse, were cleared by professional macrophages without intervention of granulocytes. No marked changes were observed in the levels of granulocyte infiltration (LY-6G) or tissue and blood cytokines, and yet almost all test bacteria were completely cleared from the lungs 1 week after exposure. In exposures containing > 10^4 live vegetative cells, at least Bc was able to produce toxins. Macrophage-induced clearance was likely inadequate and necessitated augmentation with granulocyte action. Moreover, this study revealed that for clearance and early immune effects, pure spores, as well as other substances and additives in diluted commercial products, exhibit no observable effects compared to those elicited by live, metabolically active bacteria (vegetative cells). As such, the commercial preparations tested here are not expected to be toxic following inhalational exposure to non-target mammals, including humans, and should be safe when used as intended.

Evaluation RMS	It was concluded that the commercial preparation tested (Foray 48B) is not expected to be toxic following inhalational exposure to non-target mammals, including humans, and should be safe when used as intended. Foray 48B is based on *Bt* subsp. *kurstaki* strain ABTS 351, which is closely related to Btk SA-12 and therefore the results are relevant for risk assessment of Btk SA-12.

Intraperitoneal/subcutaneous single dose

No strain specific data have been provided for first approval of SA-12.

New data 2016

For renewal of Btk SA-12, an acute intravenous toxicity study in rats with the product Thuricide SC is submitted meeting the requirements for microbial pathogenicity testing (min. 10^7 CFU/animal, intravenous administration, observation for 21 days, clearance determination,). The study is therefore considered applicable for the evaluation of possible toxicity, pathogenicity, and infectivity of Btk SA-12. Under the conditions of the study, the test item did not reveal mortality or significant adverse effects after intravenous application to rats.

From the literature search, one reference was identified, reporting on effects of Btk after intraperitoneal application to mice. Berlitz and colleagues (2012) showed, that systemic application of 200 µL of Bt 1958-2, Bt 2014-2, and the BTh Thuricide 63 at very high doses (1×10^{10} CFU/mL = 2×10^9 CFU/animal) is lethal to the animals within 6 - 24 h after injection. It is noted that the tested dosages exceed the levels recommended by EPA Microbial Pesticide Test Guideline OPPTS 885.3200 for Acute Injection Toxicity/ Pathogenicity by 2 orders of magnitude. This means that not only the exposure route but also the applied dose levels represent an unrealistic worst case.

Thus, intravenous administration of Btk SA-12 to rodents is only toxic at very high doses (2×10^9 CFU/animal) but shows no evidence of toxicity or pathogenicity at lower guideline conform dose levels (9×10^7 CFU/animal).

Reference:	**KMA 6.1.2.2/10**
Report:	[redacted] (2015c) Acute intravenous toxicity/pathogenicity study in rats (*Rattus norvegicus*) Unpublished Report No: RL1375/2015PIV-B
Guideline(s):	OPPTS 885.3200. Microbial pesticide test guidelines. Acute injection toxicity/ pathogenicity (1996)
Deviations:	Maximum mean humidity registered (74.4%) was higher than range (30 - 70%). Evaluation of the 10th of shelf control animals was not carried out due to technical problems. These deviations did not affect the study.
GLP:	Yes
Acceptability:	Acceptable
Duplication: **(if vertebrate study)**	No

Executive summary

An acute intravenous toxicity study was conducted according to OPPTS 885.3200 Microbial pesticide test guidelines. Five groups of 3 female and 3 male Wistar rats received 9×10^7 CFU/animal Thuricide SC by intravenous route (tail vein). Additionally, one negative and one witness control group were included (2 animals/sex). The experimental groups were sacrificed 1 h, and 3, 7, 14, and 21 days post application. Blood, pool of organs (brain, lymph node, kidney, lung, liver, spleen) and caecal content were analysed for MCA quantification. Control groups were observed on day 21.

Clinical signs of snout edema were observed in all animals as well as light depletion during the first 4 hours after administration. These signs did not persist after evaluation at 24 hours post application. Macroscopic changes were observed in animals of experimental and control groups. MCA > 3×10^3 CFU/mL were detected 1 h and 3 days post exposure. In organ samples of the 7 days group, 1×10^3 CFU/mL were detectable. only detected in lungs of animals analysed 1 hour and 3 days post application, but not on day 7, 14, and 21 as well as the control animals.

Thuricide SC containing the active ingredient Btk SA-12 is classified as non-pathogenic, non-toxic. Bacterial load was cleared within 14 days.

Material and Methods

Test Item

Designation	Thuricide SC

Characteristics	Suspension concentrate
Batch no.	001-14-14400
Expiration date	August 2016
Purity	5.4×10^9 CFU/mL of Btk SA-12

Test System

Species	*Rattus norvegicus*, Wistar (albino rats)
Age	8-11 weeks
Source	[redacted]
Number	38 animals, 3 males and 3 females per each experimental group; 2 males and 2 females per each control group)
Acclimatisation period	5 days

Test Conditions

Housing	2-3 rats per cage
Food	Pelleted commercial diet for the species (Nuvilab CR1, Quimtia S.A.) and filtered tap water, both provided *ad libitum*
Temperature	21.8 – 23.7°C
Photoperiod	12 h artificial light and 12 h darkness
Humidity	62.3 – 74.4%

Study Design and Methods

In-life dates	10. – 31.03.2015
Exposure	Intravenous injection in tail vein, 0.05 mL per animal resulting in 9×10^7 CFU/animal
Vehicle	Demineralized water
Post exposure observation:	21 days
Experimental treatment	The test item used was Thuricide SC containing Btk SA-12 as active substance.
	Five groups of 3 male and 3 female Wistar rats received a single dose of 0.05 mL of diluted Thuricide SC containing 1.8×10^9 CFU/mL (= 9×10^7 CFU/animal) intravenous. Two control groups, a negative control (2 female, 2 male) receiving no treatment and a sentinel control (2 female, 2 male) maintained in different environment from the experimental groups were included.
Observations	Animals were observed for signs of toxicity and mortality 1 h, and 3, 7, 14, and 21 days post-dosing. The rats were observed daily for clinical signs of toxicity/pathogenicity. Gross pathological examination was performed of the main organs (CNS, intestines, liver, kidneys, heart, spleen, lymph nodes, lung, trachea, diaphragm, thyroid, esophagus, stomach, pancreas, muscle, urinary bladder, ovaries, uterus, testicles) on terminally sacrificed animals. MCA quantification was performed in blood, pooled organs (brain, lymph nodes, lung, kidney, spleen, liver; pooled) and caecum content.

Findings

Clinical signs of edema snout were observed in all animals and prostration in 24 animals of the experimental groups during the first 4 hours after administration. However, these signs did not persist at 24 hours, and were therefore not significant in evaluation of the MCA toxicity. No mortality occurred and no macroscopic changes were detectable after single intravenous exposure to 9×10^7 CFU per animal. Microscopic findings were suggestive of an acute inflammatory reaction. Only normal gain in body weight was observed. MCA quantification

revealed recoveries of > 1.0×10^3 CFU/mL in organ pool up to 7 days post application. No recovery was observed in the samples of 14 and 21 days post application as well as in any of the control groups.

Conclusion

As no mortality or signs of toxicity or pathogenicity were observed in the rats after intravenous application of 9×10^7 CFU/animal, it is concluded, that Btk SA-12 does not warrant classification as being toxic or pathogenic. Bacterial load was cleared within 14 days.

RMS evaluation	An acute intravenous toxicity study showed no mortality or signs of toxicity or pathogenicity in rats after intravenous application of 9×10^7 CFU/animal. The study was conducted according to OPPTS 885.3200 Microbial pesticide test guidelines and considered applicable for the evaluation of possible toxicity, pathogenicity, and infectivity of Btk SA-12. It is concluded, that Btk SA-12 does not warrant classification as being toxic or pathogenic. Bacterial load was cleared within 14 days. Thus, intravenous administration of Btk SA-12 to rodents shows no evidence of toxicity or pathogenicity at dose levels > 10^7 CFU/animal.
Endpoint: Acute intravenous/ intraperitoneal infectivity	There is no evidence that Btk SA-12 acts toxic or pathogenic following intravenous administration. LD_{50} rat > 9×10^7 CFU/animal

B.6.1.2.3 Genotoxicity testing

In the DAR 2008 provided for first approval of Btk SA-12 the RMS concluded that no validated methods for genotoxicity testing of microorganism are available, and there is also a lack of relevant positive and negative controls. Therefore, the studies provided in the DAR are not included in the RAR.

New data 2016

Standard assays are not appropriate for testing the mutagenicity and genotoxicity of microorganisms. Therefore, according to Regulation (EU) No 283/2013, genotoxicity testing should be conducted only for specific metabolites. Thus, no studies using Btk SA-12 are submitted.

From the literature search covering the last 10 years and focussing on toxicity of Btk on mammals, one article was identified studying the effect of *Bacillus thuringiensis* on mouse bone marrow (Grisolia et al. 2009). After single oral application of 10^7 spores Btk, Bti or Bs to Swiss albino mice, no cytotoxic effects were seen on polychromatic (PCE) and normochromatic (NCE) erythrocytes 24 h post exposure. Thus, no genotoxic effect was detectable. Moreover, this study clearly shows that standard assays are not appropriate for testing of mutagenicity and genotoxicity of microorganisms.

Evaluation RMS	Standard assays are not appropriate for testing the mutagenicity and genotoxicity of microorganisms. Therefore, according to Regulation (EU) No 283/2013, genotoxicity testing should be conducted only for specific metabolites. For *B. thuringiensis* no relevant genotoxic metabolites are anticipated and therefore genotoxicity testing is not required for this organism.
Endpoint: Genotoxicity	No validated methods available for microorganisms.

Cited literature

Reference	**KMA 6.1.2.3/01**
Report	Grisolia, C.K., Oliveira-Filho, E.C., Ramos, F.R., Lopes, M.C., Muniz, D.H.F., Monnerat, R.G. (2009)
	Acute toxicity and cytotoxicity of *Bacillus thuringiensis* and *Bacillus sphaericus* strains on fish and mouse bone marrow
	Published report
	Ecotoxicology, 2009, 18(1):22-6
Guideline:	Not applicable
GLP:	No

Abstract

The insecticidal properties of delta-endotoxins from *Bacillus thuringiensis* (Bt) serotypes *kurstaki* and *israelensis* and crystal proteins of *Bacillus sphaericus* (Bs) serotype H5 have been used in insect control for decades. The availability of microbial toxins in biopesticides as well as in plants with incorporated protection has been increasing the concerns about biosafety. Acute toxicity to *Danio rerio* and cytotoxicity on mouse bone marrow cells and peripheral erythrocytes of *Oreochromis niloticus* were tested with *Bt israelensis*, *Bt kurstaki* and Bs H5 strains. The concentration and dose tested were 10^6 and 10^8 spores/mL, respectively. Neither lethality nor effects on mouse bone marrow were promoted by any strain. In necrosis-apoptosis study on peripheral erythrocytes of *O. niloticus* an increased frequency of necrotic cells caused by exposure to strains of *B. thuringiensis* was found. Exposure to *B. sphaericus* did not show cytotoxic effects in either tested system. None of the strains studied induced apoptosis in contrast with the chemical controls.

Material and Methods

Groups of 6 Swiss mice (males and females) were dosed with 100 µL test solution of *B. thuringiensis* serotypes *kurstaki; B. thuringiensis* serotypes *israelensis, B. sphaericus* serotype H5 or vehicle via gavage. CFU of all Bacillus administered was each at 10^8 spores /mL and filtered water served as vehicle. Animals were sacrificed 24 hours post exposure and bone marrow preparation for polychromatic (PCE) and normochromatic (NCE) erythrocyte identification according to Schmid[9] (1975) was performed. Blood smears were prepared, Giemsa stained and 10000 cells per animal were scored.

Findings

No lethality or effects on cell proliferation in mouse bone marrow by the three strains tested compared to control were detectable. Thus, no cytotoxicity was observed.

Table 5.2.3.1-1: Means and percentage of NCEs in mouse bone marrow cells

Strains/treatments	Mean of NCE ± SD	% of NCE	*t*-Test (*P*)
Control	622 ± 95	38.3	–
Bt *kurstaki* 48 h	564 ± 72	36.0	0.5416
Bt *kurstaki* 96 h	617 ± 110	38.1	0.9564
Bt *israelensis* 48 h	722 ± 180	41.9	0.3909
Bt *israelensis* 96 h	578 ± 120	36.6	0.7163
Bs H5 48 h	441 ± 98	30.6	0.0676
Bs H5 96 h	475 ± 87	31.3	0.0734

* $P > 0.05$, no significant

Conclusion

In the mouse bone marrow assay, no evidence on toxicity of Btk, Bti or Bs at a dose level of 10^7 CFU is given.

[9] Schmid W (1975) The micronucleus test. Mutat Res 31:9–15

Evaluation RMS	No remarks

B.6.1.2.4 Cell culture study

New data 2016

Btk is not an intracellular replicating microorganism. Thus, according to Regulation (EU) No 283/2013, cell culture studies are not required.

Evaluation RMS	Btk is not an intracellular replicating microorganism and therefore cell culture studies are not required.
Endpoint: Cell culture study	Btk SA-12 is not an intracellular replicating microorganism; cell culture studies are not required.

B.6.1.2.5 Information on short-term toxicity and pathogenicity

No strain specific data have been provided for first approval of SA-12.

Information from open literature

Hadley *et al*. (1987) administered Dipel (subsp. *kurstaki* HD-1) in the diet for 5 months at a dose of 500 mg/kg/day (approximately 10^{12} spores per day) to six sheep. Two sheep developed diarrhea in week 2 which continued through test week 3. The sheep in this group generally had chronic loose stools. No treatment-related effect was seen on weight gain or clinical chemistry parameters nor was significant gross clinical changes observed. All sheep had blood cultures positive for *B. thuringiensis* and one was positive for presence of *B. thuringiensis* in the spleen. Several incidental lesions were observed, however the only lesion that may have been associated with the treatment was a moderate to marked lymphoid hyperplasia in Peyer's patches of caecum and colon in two out of the six sheep. However, it was not considered to be clinically significant. The study gave no indication that *B. thuringiensis* is a pathogen in sheep following oral ingestion of large daily doses for five months.

New data 2016

Information provided for first approval are considered acceptable to cover current requirements. In addition, acute toxicity studies did not reveal any signs of toxicity or pathogenicity. Therefore, no new studies are submitted for renewal of the strain according to Regulation (EC) No 1107/2009.

No additional information on short term toxicity or pathogenicity of Btk is reported in open peer-reviewed literature (please refer to the literature research report Seehase 2016; KMA 6.1.1/07).

Thus, there is no evidence that Btk SA-12 acts toxic or pathogenic following short-term exposure.

Health effects after repeated oral exposure

No further testing is required, since the acute oral study as well as acute inhalation and i.v. studies provided for re-approval of Btk SA-12 did not show any toxicological effects of the strain. In addition, a short-term toxicity study according to OECD 408 with the closely related strain Btk SA-11 did not reveal in any adverse effects in the test animals.

Evaluation RMS	No studies were presented on Btk SA-12 or formulated products for first approval or renewal of Btk SA-12. No further testing on repeated oral exposure is required, since the acute oral and inhalation studies provided for of Btk SA-12 did not show

	any toxicological effects of the strain.

Health effects after repeated inhalatory exposure

It is referred to data provided for first approval of Btk SA-12. No further testing is required, since the acute inhalation study as well as acute oral and i.v. studies provided for re-approval of Btk SA-12 did not show any toxicological effects on the strain.

New data 2016

Data provided for first approval are considered acceptable to cover current requirements. Apart from results of the literature search, no substantial new information is submitted for renewal of Btk SA-12 according to Regulation (EC) No 1107/2009.

In the literature search covering the last 10 years and focussing toxicity or pathogenicity of Btk on mammals, one article was identified concerning repeated inhalatory exposure.

A non-GLP study in mice not following any guideline by Barfod et al (2010) showed no increase of inflammatory cells in bronchoalveolar fluid and no changes in lung function parameters and thus no airway irritation 70 days after repeated exposure to Bt-containing MPCPs. However, an interstitial lung inflammation was detected in 3 out of 17 mice after treatment with Vectobac® containing the active ingredient *Bt israelensis*, whereas less significant effects were observed in mice treated with Dipel® containing the MPCA *Bt kurstaki*. The subchronic inflammation observed in this study, was most likely due to the prolonged presence of Bt spores or other product residues in the lungs, triggering and maintaining the inflammatory response. The formulated MPCP contained only about 2% spores and 98% other ingredients according to manufacturer.

Thus, there is no evidence that Btk may cause serious health effects after repeated inhalatory exposure in mammals.

Evaluation RMS	No studies were presented on Btk SA-12 or formulated products for first approval or renewal of Btk SA-12. No further testing on repeated inhalatory exposure is required, since the acute inhalation study and the i.p. study provided for first approval of Btk SA-12 did not show any toxicological effects on the strain.

Cited references:

Reference	**KMA 6.1.2.2/05**
Report	Barfod, K.K., Poulsen, S.S., Hammer, M., Larsen, S.T. (2010) Sub-chronic lung inflammation after airway exposures to *Bacillus thuringiensis* biopesticides in mice. Published report BMC Microbiol 2010, 3, 10:233
Guideline:	Not applicable
GLP:	No

Abstract

BACKGROUND: The aim of the present study was to assess possible health effects of airway exposures to Bacillus thuringiensis (Bt) based biopesticides in mice. Endpoints were lung inflammation evaluated by presence of inflammatory cells in bronchoalveolar lavage fluid (BALF), clearance of bacteria from the lung lumen and histological alterations of the lungs. Hazard identifications of the biopesticides were carried out using intratracheal (i.t.) instillation, followed by an inhalation study. The two commercial biopesticides used were based on the Bt. subspecies kurstaki and israelensis, respectively. Groups of BALB/c mice were i.t instilled with one bolus (3.5 ×

10^5 or 3.4×10^6 colony forming units (CFU) per mouse) of either biopesticide. Control mice were instilled with sterile water. BALFs were collected and the inflammatory cells were counted and differentiated. The BALFs were also subjected to CFU counts.

RESULTS: BALF cytology showed an acute inflammatory response dominated by neutrophils 24 hours after instillation of biopesticide. Four days after instillation, the neutrophil number was normalised and inflammation was dominated by lymphocytes and eosinophils, whereas 70 days after instillation, the inflammation was interstitially located with few inflammatory cells present in the lung lumen. Half of the instilled mice had remaining CFU recovered from BALF 70 days after exposure. To gain further knowledge with relevance for risk assessment, mice were exposed to aerosols of biopesticide one hour per day for 2×5 days. Each mouse received 1.9×10^4 CFU *Bt israelensis* or 2.3×10^3 CFU *Bt kurstaki* per exposure. Seventy days after end of the aerosol exposures, 3 out of 17 mice had interstitial lung inflammation. CFU could be recovered from 1 out of 10 mice 70 days after exposure to aerosolised *Bt kurstaki*. Plethysmography showed that inhalation of Bt aerosol did not induce airway irritation.

CONCLUSIONS: Repeated low dose aerosol exposures to commercial Bt based biopesticides can induce sub-chronic lung inflammation in mice, which may be the first step in the development of chronic lung diseases. Inhalation of Bt aerosols does not induce airway irritation, which could explain why workers may be less inclined to use a filter mask during the application process, and are thereby less protected from exposure to Bt spores.

Material and Methods

Bacterial suspensions were prepared from the commercially available insecticides Vectobac® (*Bt israelensis*) and Dipel® (*Bt kurstaki*), both from Valent Biosciences (Sumitomo Chemical Agro Europe, Lyon, France).

Groups of nine BALB/c mice (Taconic M&B, Ry, Denmark) were head-only exposed to 5×10^6 CFU Vectobac or 3.5×10^5 CFU Dipel for 60 min/5 days per week for two weeks with 2-day break in between in a whole body plethysmograph. Respiratory parameters including respiratory rate, time of break or time of pause, were collected during the 60 min exposure time to assess airway irritation.

Findings

In the mice exposed by inhalation to Dipel® aerosols, 1 out of 10 mice had CFU recovered (630 CFU/BALF). No CFU was recovered from mice exposed to Vectobac® aerosol. Plethysmography showed that inhalation of Bt aerosol did not induce airway irritation. Histopathological evaluation revealed a slight interstitial inflammation after Vectobac® instillation. Instillation of Dipel® resulted in fewer and less intense changes. One mouse was excluded from further analyses due to leukemia. In 3 of the remaining 17 mice, some patches of interstitial inflammation were observed 70 days after end of the repeated exposures to Vectobac®, whereas exposure to Dipel® gave rise to less significant effects.

Conclusion

Repeated low dose aerosol exposures to commercial Bt based biopesticides induced sub-chronic lung inflammation in mice, which may be the first step in the development of chronic lung diseases. Additionally, the sub-chronic inflammation observed in the presented study, was most likely due to the prolonged presence of Bt spores or other product residues in the lungs, triggering and maintaining the inflammatory response. This should be seen in the light that the formulated biopesticides contains only about 2% spores and 98% other ingredients according to manufacturer which makes long term inhalation studies using the final formulated biopesticide important. Therefore, alternative inoculums or controls, including spore free or heat-inactivated biopesticide or specific excipients/ additives should also be studied for biological effect. Moreover, inhalation of Bt aerosols does not induce airway irritation, which could explain why workers may be less inclined to use a filter mask during the application process and are thereby less protected from exposure to Bt spores.

Evaluation RMS	No remarks

B.6.1.2.6 Proposed treatment: first aid measures, medical treatment

No specific treatment after contact with *Bacillus thuringiensis* is required since *Bacillus thuringiensis* does not infect domestic animals and man. Moreover, Btk SA-12 is not multi-resistant to commonly used antibiotics and thus, medical treatment in the rare case of an infection is ensured.

In case of accidental direct contact with *Bacillus thuringiensis* the producer states the below listed "first aid instructions" (Safety Data Sheet, Doc M-MP, Section 4, Point 4.4).

First aid measures:	
Skin contact:	Wash with plenty of soap and water, including hair and under fingernails. Do not apply any medicating agent except on the advice of a physician. Remove contaminated clothing and decontaminate prior to use.
Eye contact:	Immediately wash eyes with a large amount of running water. Hold eyelids apart to rinse the entire surface of the eyes and lids. Do not apply any medicating agent except on the advice of a physician.
Inhalation:	Move victim from contaminated area to fresh air. Apply artificial respiration if necessary.
Ingestion:	If victim is fully conscious, immediately give large amounts of water to drink and induce vomiting. Never give anything by mouth to an unconscious person.

B.6.1.3 Toxicity studies on metabolites and relevant impurities

Not relevant

B.6.1.4 Summary and conclusions of Tier I studies

No toxicity or infectivity was noted in experimental studies upon oral, respiratory and intravenous exposure even to exceedingly high dose levels (Table 6.1.4-1). Upon administration of extremely high dose levels by invasive routes mortality occurred in laboratory animals. However, lower doses applied by these routes caused no adverse effects. Taking together the results of these experimental studies, of epidemiological and occupational evidence and the experience from several decades of safe application of Btk-based plant protection products it is appropriate to state that there is no concern with regard to human health.

Table 6.1.4-1: Overview of experimental studies on oral, respiratory, intraperitoneal toxicity or genotoxicity after exposure to *Bacillus thuringiensis* subsp. *kurstaki* SA-12

Study type	Species	Test item	Dose level	Findings	References
Acute oral toxicity	Rat	Btk SA-12 [pSB337]	1.2×10^8 CFU/animal	No adverse effect, No infectivity	[redacted] 1992 KMA 6.1.2.2/01
Acute oral toxicity	Rat	Costar Technical Concentrate (Btk SA-12)	5050 mg/kg bw	None	[redacted] 1999a KMA 6.1.2.2/02
Acute oral toxicity	Rat	Btk Thuricide SC (Btk SA-12)	5.4×10^8 CFU/animal	No adverse effects, clearance 7 days	[redacted] 2015a KMA 6.1.2.2/03
Acute respiratory toxicity	Rat	Btk Thuricide SC (Btk SA-12)	1.35×10^8 CFU/animal	No adverse effects, clearance 7 days	[redacted], 2015b Vol. 3 MA, B.6.1.2.2
Acute intravenous toxicity/pathogenicity	Rat	Btk Thuricide SC (Btk SA-12)	9×10^7 CFU/animal	No symptoms of toxicity or pathogenicity, clearance 14 days	[redacted] 2015c Vol. 3 MA, B.6.1.2.2/

B.6.2 Tier II

B.6.2.1 Specific toxicity, pathogenicity and infectiveness studies

No adverse effects were observed in acute toxicity studies with Btk SA-12. Therefore, no further studies on specific toxicity, pathogenicity and infectiveness are required.

From the literature search, no reference was identified, reporting specific toxicity, pathogenicity or infectiveness of Btk after various routes of exposure. Please refer to the literature search report for detailed information on the search strategy (Seehase 2016, Vol. 3MA, B.6.3).

B.6.2.2 *In vivo* studies in somatic cells

Data provided for first approval are considered acceptable to cover current requirements. No new *in vivo* study in somatic cells was conducted and is not considered necessary. No substantial new information is required for renewal of the strain according to Regulation (EC) No 1107/2009.

B.6.2.3 Genotoxicity – *In vivo* studies in germ cells

No indications of genotoxicity are known for Btk. Therefore, studies in genotoxic effects in germ cells were not considered necessary.

B.6.2.4 Summary and conclusions of Tier II studies

No further studies are required. Tier II were not considered necessary by the RMS.

B.6.3 References relied on

Several literature review reports have been provided according to the guidance of EFSA (Guidance of EFSA: Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009. EFSA Journal 2011;9(2):2092). The aim of these reports was to provide a global overview of peer-reviewed literature concerning potential side effects of *B. thuringiensis* subsp. *kurstaki* strain SA-12.

Overview of literature reports provided according to the guidance of EFSA

Data point	Author	Year	Title	Section of RMS evaluation
KMA 2.7/12 & 3.5/06	Süß, J.	2016	Literature review on *B. thuringiensis* subsp. *kurstaki* strain SA-12: Biological properties	Vol. 3MA, B.2.10
KMA 6.1.1/07	Seehase, S.	2016	Literature review on *B. thuringiensis* subsp. *kurstaki* strain SA-12: Toxicology	Vol. 3MA, B.6.3
KMA 7.1/01	Cornelese, A.	2016a	Literature review on *B. thuringiensis* subsp. *kurstaki* strain SA-12 and metabolites: Residues in or on treated products, food and feed	Vol. 3MA, B.7.4
KMA 8.1/10	Cornelese, A.	2016b	Literature review on *B. thuringiensis* subsp. *kurstaki* strain SA-12: Fate and behaviour in the environment	Vol. 3MA, B.8.3
KMA 9/01	Schöbinger, U.	2016	Literature review on *B. thuringiensis* subsp. *kurstaki* strain SA-12: Effects on non-target organisms	Vol. 3MA, B.9.8

Literature review on *B. thuringiensis* subsp. *kurstaki* strain SA-12: Toxicology (Seehase, 2016)

RMS comments on the literature search: "Literature review on *B. thuringiensis* subsp. *kurstaki* strain SA-12: Toxicology (Seehase, 2016; submitted in Point KMA 6.1)	The review was made in order to identify scientific peer-reviewed open literature on the active substance *Bacillus thuringiensis* subsp. *kurstaki* SA-12 and its metabolites, which may affect the assessment on human health, animal health and/or the environment. The search strategy was based on a multi-concept approach. **Relevance criteria** • Property investigated was relevant for data requirements of Regulation (EC) 1107/2009 • Identification of the test species as *Bacillus thuringiensis* spp. *kurstaki* • Subject relevant for toxicological considerations • Test species relevant to the toxicological assessment • Route of administration / exposure relevant for assessment • Endpoint relevant for assessment • Clinical cases and follow-up studies • In the case of reports on known *Bacillus thuringiensis* pathogens, is there any relevance for *Bacillus thuringiensis* spp. *kurstaki* used as microbial pest control agent? • Metabolites or toxins of toxicological concern produced by *Bacillus thuringiensis* spp. *kurstaki* **Database searched** A search was conducted using the DIMDI database provided by the German Institute of Medical Documentation and comprised of searches in MEDLINE, BIOSIS, CAB and SCISEARCH databases

	Search methods Search strategy aimed to find all recent (from 2006 onwards) references relevant addressing the potential pathogenicity, infectivity and toxicity of *B. thuringiensis* subsp. *kurstaki*, including toxic products and metabolites. For the selection of the keywords and the search strategy, a recent EFSA supporting publication on Literature search and data collection on risk assessment for human health for microorganisms used as plant protection products[10] was considered. **Results of the study selection process** In total 46 references were retrieved and first subjected to a *rapid assessment* based on title and the abstract. After the *rapid assessment* 13 references were identified as being potentially relevant and subjected to a *detailed assessment* of full-text documents. Summary records that appeared to be relevant passed to a second step in which a detailed assessment of full text documents was conducted. In total 11 references were identified as being potentially relevant and are summarised under the respective data points. One article was not available in full text and could not be taken into account. The other article Mezzomo BP, Miranda-Vilela AL, de Souza Freire I, Barbosa LCP, Portilho FA & Grisolia CK. 2015a. Hematotoxicity of *Bacillus thuringiensis* as Spore-crystal Strains Cry1Aa, Cry1Ab, Cry1Ac or Cry2Aa in Swiss Albino Mice. Journal of Hematology & Thromboembolic Diseases 1(1) has been identified as not relevant upon full text analysis. In our opinion, the study by Mezzomo et al. does not provide new knowledge regarding risk assessment of registered and approved live *Bacillus thuringiensis* microbiological plant protection agents. We reach this conclusion mainly because intraperitoneal exposure (or intravenous exposure), as studied here, is not considered a relevant exposure route for humans. The levels of exposure in this study to purified spore crystals were extremely high as compared to environmental conditions. Furthermore, the RMS agrees with the applicant due to the lacks in experimental procedure, the presented study is considered not relevant.
Conclusion	The literature search regarding data of toxicology was accepted as valid, both regarding inclusion of databases and use of search terms. By the searches for the potential pathogenicity, infectivity and toxicity of *B. thuringiensis* subsp. *kurstaki*, including toxic products and metabolites 11 references were finally considered relevant and reliable and are included in the dossier, respectively.

[10] *Evelyn Hackl et al. (2015).* Literature search and data collection on RA for human health for microorganisms used as plant protection products *EFSA supporting publication 2015:EN-801. 173 pp*

Data point	Author(s)	Year	Title Owner Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KMA 6.1.1 /01	Glare, T.R., O'Callaghan, M.	2000	BACILLUS THURINGIENSIS: BIOLOGY, ECOLOGY AND SAFETY Bacillus thuringiensis: Biology, Ecology and Safety, Glare, T.R., O'Callaghan, M. (eds.) John Wiley & Sons, Ltd., 2000 Report-no. not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 6.1.1 /02	WHO	1999	MICROBIAL PEST CONTROL AGENT BACILLUS THURINGIENSIS Environmental Health Criteria 217, WHO Report no. not applicable GLP/GEP: no Published: Yes	no	no	not protected	-	DAR 2008
KMA 6.1.1 /03	Siegel, J.P.	2001	THE MAMMALIAN SAFETY OF BACILLUS THURINGIENSIS-BASED INSECTICIDES Journal of Invertebrate Pathology 77, 13-21, 2001 Report-no. not applicable GLP/GEP: no Published: yes	yes	no	not protected	-	DAR 2008
KMA 6.1.1 /04	McClintock, J.T., Schaffer, C.R., Sjobald, R.D.	1995	A COMPARATIVE REVIEW OF THE MAMMALIAN TOXICITY OF BACILLUS THURINGIENSIS-BASED PESTICIDES Pestic. Sci. 45:95-105 Report-no. not applicable GLP/GEP: no Published: yes	yes	no	not protected	-	DAR 2008
KMA 6.1.1 /05	Wilcks, A., Hansen, B.M., Hendriksen, N.B., Licht, T.R.	2006b	FATE AND EFFECT OF INGESTED BACILLUS CEREUS SPORES AND VEGETATIVE CELLS IN THE INTESTINAL TRACT OF HUMAN-FLORA-ASSOCIATED RATS FEMS Immunol Med Microbiol 46:70-77 Report-no. not applicable GLP/GEP: no Published: Yes	yes	no	not protected	-	DAR 2008
KMA 6.1.1 /06	Itoh, T., Arai, T., Hirata, I.	1991	ENTEROPATHOGENICITY OF BACILLUS THURINGIENSIS FOR HUMANS Shokubutsu Boeki 45:18-22 Report-no. not applicable GLP/GEP: no Published: yes	yes	no	not protected	-	DAR 2008

Data point	Author(s)	Year	Title Owner Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KMA 6.1.1 /07	Seehase, S.	2016	LITERATURE REVIEW ON BACILLUS THURINGIENSIS SUBSP. KURSTAKI SA-12: TOXICOLOGY Certis USA LLC GAB Consulting GmbH, Stade, Germany Report-no.: 2281384-MA-05-01_SA-12 GLP/GEP: no Published: no	no	yes	Protected	Certis USA	New data for active ingredient, not previously submitted nor evaluated
KMA 6.1.1 /08	Berlitz, D.L., Giovenardi, M., Charles, J.F., Fiuza, L.M.	2012	TOXICITY INTRAPERITONEAL AND INTRAGASTRIC ROUTE OF BACILLUS THURINGIENSIS AND MELIA AZEDARACH IN MICE not applicable Arq. Inst. Biol., 79(4), 511-517 GLP/GEP: no Published: yes	yes	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated
KMA 6.1.1 /09	Wilcks, A., Hansen, B.M., Hendriksen, N.B., Licht, T.R.	2006a	PERSISTENCE OF BACILLUS THURINGIENSIS BIOINSECTICIDES IN THE GUT OF HUMAN-FLORA-ASSOCIATED RATS FEMS Immunol Med Microbiol, 48, 410-418 Report-no.: not applicable GLP/GEP: no Published: yes	yes	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated
KMA 6.1.1 /10	Hansen, V.M., Eilenberg, J., Madsen, A.M.	2010	OCCUPATIONAL EXPOSURE TO AIRBORNE BACILLUS THURINGIENSIS KURSTAKI HD1 AND OTHER BACTERIA IN GREENHOUSES AND VEGETABLE FIELDS. Biocontrol Science and Technology, 20(6), 605-619 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated
KMA 6.1.1 /11	Levin, D.B.	2009	HUMAN HEALTH EFFECTS RESULTING FROM EXPOSURE TO BACILLUS THURINGIENSIS APPLIED DURING INSECT CONTROL PROGRAMS Use of Microbes for Control and Eradication of Invasive Arthropods, 6: 291-303 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated

Data point	Author(s)	Year	Title Owner Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KMA 6.1.1 /12	Baelum, J., Larsen, P., Doekes, G., Sigsgaard, T.	2012	HEALTH EFFECTS OF SELECTED MICROBIOLOGICAL CONTROL AGENTS. A 3-YEAR FOLLOW-UP STUDY Ann Agric Environ Med, 19(4), 631-636 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated
KMA 6.1.1.1 /01	Warren, R.E., Rubenstein, D., Ellar, D.J., Kramer, J.M., Gilbert, R.J.	1984	BACILLUS THURINGIENSIS VAR. ISRAELENSIS: PROTOXIN ACTIVATION AND SAFETY Lancet 24:678-679 Report-no.: not applicable GLP/GEP: no Published: yes	yes	no	not protected	-	DAR 2008
KMA 6.1.1.1 /02	Damgaard, P.H., Granum, P.E., Bresciania, J., Torregrossa, M.V., Eilenberg, J., Valentino, L.	1997	CHARACTERIZATION OF BACILLUS THURINGIENSIS ISOLATED FROM INFECTIONS IN BURN WOUNDS FEMS Immunol. Med. Microbiol. 18:47-53 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 6.1.1.1 /03	Hernandez, E., Ramisse, F., Ducoureau, J., Cruel, T., Cavallo, J.	1998	BACILLUS THURINGIENSIS SUBSP. KONKUKIAN (SEROTYPE H34) SUPERINFECTION: CASE REPORTS AND EXPERIMENTAL EVIDENCE OF PATHOGENICITY IN IMMUNOSUPPRESSED MICE J. Clin. Microbiol. 36:2138-2139 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 6.1.1.1 /04	Hernandez, E., Ramisse, F., Cruel, T., Vagueresse, R., Cavallo, J.	1999	BACILLUS THURINGIENSIS SEROTYPE H34 ISOLATED FROM HUMANS AND INSECTICIDAL STRAINS SEROTYPES 3A3B AND H14 CAN LEAD TO DEATH OF IMMUNOCOMPETENT MICE AFTER PULMONARY INFECTION FEMS Immunol. Med. Microbiol. 24:43-47 Report-no.: not applicable GLP/GEP: no Published: yes	yes	no	not protected	-	DAR 2008

Data point	Author(s)	Year	Title Owner Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KMA 6.1.1.1 /05	Jackson, S.G., Goodbrand, R.B., Ahmed, R. and Kasatiya, S.	1995	BACILLUS CEREUS AND BACILLUS THURINGIENSIS ISOLATED IN A GASTROENTERITIS OUTBREAK INVESTIGATION Letters in Appl. Microbiol. 21, p. 103-105, 1995 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 6.1.1.1 /06	Green, M., Heumann, M., Sokolow, R., Foster, L.R., Bryant, R., Skeels, M.	1990	PUBLIC HEALTH IMPLICATIONS OF THE MICROBIAL PESTICIDE BACILLUS THURINGIENSIS: AN EPIDEMIOLOGICAL STUDY, OREGON, 1985-86 Am. J. Publ. Health 80:848-852 Report no. not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 6.1.1.1 /07	Callegan, M.C., Cochran, D.C., Kane, S.T., Ramadan, R.T., Chodosh, J., McLean, C., Stroman, D.W.	2006	VIRULENCE FACTORS PROFILES AND ANTIMICROBIAL SUSCEPTIBILITIES OF OCULAR BACILLUS ISOLATES Curr. Eye Res. 31:693-702 Report no. not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 6.1.1.1 /08	Samples, J.R., Buettner, H.	1983	CORNEAL ULCER CAUSED BY A BIOLOGICAL INSECTICIDE (BACILLUS THURINGIENSIS) Am. J. Ophthalmol. 95:258-260 Report no. not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 6.1.1.2 /01	Dively, C.A.	2005	LONG TERM EXPOSURE OF BT TO EMPLOYEES DURING PRODUCTION Certis USA LLC, Columbia Report no. not applicable GLP/GEP: no Published: yes	no	yes	protected	Certis USA	DAR 2008
KMA 6.1.1.2 /02	Doak, B.	2016	BTZ MEDICAL VERIFICATION Certis USA LLC, not stated [redacted] Report-no.: not applicable GLP/GEP: no Published: no	no	yes	protected	Certis USA	New data for active ingredient, not previously submitted nor evaluated

Data point	Author(s)	Year	Title Owner Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KMA 6.1.1.2 /03	Baelum, J., Larsen, P., Doekes, G., Sigsgaard, T.	2012	HEALTH EFFECTS OF SELECTED MICROBIOLOGICAL CONTROL AGENTS. A 3-YEAR FOLLOW-UP STUDY Ann Agric Environ Med, 19(4), 631-636 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated
KMA 6.1.1.2 /04	Hansen, V.M., Eilenberg, J., Madsen, A.M.	2010	OCCUPATIONAL EXPOSURE TO AIRBORNE BACILLUS THURINGIENSIS KURSTAKI HD1 AND OTHER BACTERIA IN GREENHOUSES AND VEGETABLE FIELDS. Biocontrol Science and Technology, 20(6), 605-619 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated
KMA 6.1.1.3 /01	Bernstein, I.L., Bernstein, J.A., Miller, M., Tierzieva, S., Bernstein, D.I., Lummus, Z., Selgrade, M.K., Doerfler, D.L., Seligy, V.L.	1999	IMMUNE RESPONSES IN FARM WORKERS AFTER EXPOSURE TO BACILLUS THURINGIENSIS PESTICIDES Environ. Health Perspec. 107:575-582 Report no. not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 6.1.1.3 /02	WHO	1999	MICROBIAL PEST CONTROL AGENT BACILLUS THURINGIENSIS Environmental Health Criteria 217, WHO Report no. not applicable GLP/GEP: no Published: Yes	no	no	not protected	-	DAR 2008
KMA 6.1.1.3 /03	Doekes, G., Larsen, P., Sigsgaard, T., Baelum, J.	2004	IGE SENSITIZATION TO BACTERIAL AND FUNGAL BIOPESTICIDES IN A COHORT OF DANISH GREENHOUSE WORKERS: THE BIOGART STUDY Am. J. Indust. Med. 46:404-407 Report no. not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008

Data point	Author(s)	Year	Title Owner Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KMA 6.1.1.3 /04	McClintock, J.T., Schaffer, C.R., Sjobald, R.D.	1995	A COMPARATIVE REVIEW OF THE MAMMALIAN TOXICITY OF BACILLUS-THURINGIENSIS BASED PESTICIDES Pestic. Sci. 45:95-105 Report no. not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 6.1.1.3 /05	Hansen, V.M., Eilenberg, J., Madsen, A.M.	2010	OCCUPATIONAL EXPOSURE TO AIRBORNE BACILLUS THURINGIENSIS KURSTAKI HD1 AND OTHER BACTERIA IN GREENHOUSES AND VEGETABLE FIELDS. Biocontrol Science and Technology, 20(6), 605-619 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated
KMA 6.1.1.3 /06	Baelum, J., Larsen, P., Doekes, G., Sigsgaard, T.	2012	HEALTH EFFECTS OF SELECTED MICROBIOLOGICAL CONTROL AGENTS. A 3-YEAR FOLLOW-UP STUDY Ann Agric Environ Med, 19(4), 631-636 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated
KMA 6.1.1.4 /01	Pearce, M., Habbick, B., Williams, J., Eastman, M., Newman, M.	2002a	THE EFFECTS OF AERIAL SPRAYING WITH BACILLUS THURINGIENSIS KURSTAKI ON CHILDREN WITH ASTHMA Can. J. Public Health 93:21-25 Report no. not applicable GLP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 6.1.1.4 /02	Petrie, K., Thomas, M., Broadbent, E.	2003	SYMPTOM COMPLAINTS FOLLOWING AERIAL SPRAYING WITH BIOLOGICAL INSECTICIDE FORAY 48B New Zealand Med. J. 116 (1170):1-7 Report no. not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 6.1.1.4 /03	Pearce, M., Behie, G., Chappell, N.	2002 b	THE EFFECTS OF AERIAL SPRAYING WITH BACILLUS THURINGIENSIS KURSTAKI ON AREA RESIDENTS Env. Health Rev. Spring 2002:19-22 Report no. not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008

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KMA 6.1.1.4 /04	Green, M., Heumann, M., Sokolow, R., Foster, L.R., Bryant, R., Skeels, M.	1990	PUBLIC HEALTH IMPLICATIONS OF THE MICROBIAL PESTICIDE BACILLUS THURINGIENSIS: AN EPIDEMIOLOGICAL STUDY, OREGON, 1985-86 Am. J. Publ. Health 80:848-852 Report no. not applicable GLP/GEP: no Published: yes	no	no	not protected	-	DAR 2008
KMA 6.1.2.1 /01	Baelum, J., Larsen, P., Doekes, G., Sigsgaard, T.	2012	HEALTH EFFECTS OF SELECTED MICROBIOLOGICAL CONTROL AGENTS. A 3-YEAR FOLLOW-UP STUDY Ann Agric Environ Med, 19(4), 631-636 Report-no.: not applicable GLP/GEP: no Published: yes	no	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated
KMA 6.1.2.2 /01	[redacted]	1992	ACUTE ORAL TOXICITY STUDY OF SA12[PSB337] IN RATS Certis USA LLC, Colombia [redacted] Report no. G-7409.210 GLP: yes Published: no	yes	no	Not protected	Certis USA	DAR 2008
KMA 6.1.2.2 /02	[redacted]	1999a	COSTAR TECHNICAL CONCENTRATE – ACUTE ORAL TOXICITY STUDY IN RATS Certis USA LLC, Colombia [redacted] Report no. 5397-99 GLP: yes Published: no	yes	no	Not protected	Certis USA	DAR 2008
KMA 6.1.2.2 /03	[redacted]	2015a	THURICIDE SC - ACUTE ORAL TOXICITY/PATHOGENICITY STUDY IN RATS (RATTUS NORVEGICUS) Certis USA LLC, 1372/2015PO, RL1372/2015PO-B [redacted] GLP: yes Published: no	yes	yes	protected	Certis USA	New data for active ingredient, not previously submitted nor evaluated

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KMA 6.1.2.2 /04	Wilcks, A., Hansen, B.M., Hendriksen, N.B., Licht, T.R.	2006a	PERSISTENCE OF BACILLUS THURINGIENSIS BIO-INSECTICIDES IN THE GUT OF HUMAN-FLORA-ASSOCIATED RATS FEMS Immunol Med Microbiol, 48, 410-418 Report-no.: not applicable GLP/GEP: no Published: yes	yes	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated
KMA 6.1.2.2 /05	Berlitz DL, Gionedardi M, Charles J-F, Finza LM.	2012	TOXICITY INTRAPERITONEAL AND INTRAGASTRIC ROUTE OF BACILLUS THURINGINSIS AND MELIA AZEDARACH IN MICE Arquivos do Instituto Biologico, 79(4):511-517 Report-no.: not applicable GLP/GEP: no Published: yes	yes	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated
KMA 6.1.2.2 /06	Wilcks, A., Hansen, B.M., Hendriksen, N.B., Licht, T.R.	2006 b	FATE AND EFFECT OF INGESTED BACILLUS CEREUS SPORES AND VEGETATIVE CELLS IN THE INTESTINAL TRACT OF HUMAN-FLORA-ASSOCIATED RATS FEMS Immunol Med Microbiol 46:70-77 Report no: not applicable GLP/GEP: no Published: yes	yes	no	not protected	-	DAR 2008
KMA 6.1.2.2 /07	[redacted]	2015 b	THURICIDE SC - ACUTE PULMONARY TOXICITY/PATHOGENICITY STUDY IN RATS (RATTUS NORVEGICUS) Certis USA LLC, 1398/2015PP, RL1398/2015PP-B [redacted] GLP: yes Published: no	yes	yes	protected	Certis USA	New data for active ingredient, not previously submitted nor evaluated
KMA 6.1.2.2 /08	Barfod, K.K., Poulsen, S.S., Hammer, M., Larsen, S.T.	2010	SUB-CHRONIC LUNG INFLAMMATION AFTER AIRWAY EXPOSURES TO BACILLUS THURINGIENSIS BIOPESTICIDES IN MICE BMC Microbiology, 3, 10:233 Report-no.: not applicable GLP/GEP: no Published: yes	yes	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated

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KMA 6.1.2.2 /09	Tayabali, A.F., Nguyen, K.C., Seligy, V.L.	2011	EARLY MURINE IMMUNE RESPONSES FROM ENDOTRACHEAL EXPOSURES TO BIOTECHNOLOGY-RELATED BACILLUS STRAINS Toxicological & Environmental Chemistry, 93(1), 314-331 Report-no.: not applicable GLP/GEP: no Published: yes	yes	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated
KMA 6.1.2.2 /10	[redacted]	2015c	THURICIDE SC - ACUTE INTRAVENOUS TOXICITY/PATHOGENICITY STUDY IN RATS (RATTUS NORVEGICUS) Certis USA LLC, 1375/2015PIV, RL1375/2015PIV-B [redacted] GLP: yes Published: no	yes	yes	protected	Certis USA	New data for active ingredient, not previously submitted nor evaluated
KMA 6.1.2.3 /01	Grisolia, C.K., Oliveira-Filho, E.C., Ramos, F.R., Lopes, M.C., Muniz, D.H.F., Monnerat, R.G.	2009	ACUTE TOXICITY AND CYTOTOXICITY OF BACILLUS THURINGIENSIS AND BACILLUS SPHAERICUS STRAINS ON FISH AND MOUSE BONE MARROW. Ecotoxicology, 18(1), 22-26 Report-no.: not applicable GLP/GEP: no Published: yes	yes	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated
KMA 6.1.2.5 /01	Hadley, W.M., Burchiel, S.W., McDowell, T.D., Thilsted, J.P., Hibbs, C.M., Whorton, J.A., Day, P.W., Friedman, M.B., Stoll, R.E.	1987	FIVE-MONTH ORAL (DIET) TOXICITY/INFECTIVITY STUDY OF BACILLUS THURINGIENSIS INSECTICIDES IN SHEEP Fund. Appl. Toxicol 8:236-242 Report no. not applicable GLP/GEP: no Published: yes	yes	no	not protected	-	DAR 2008
KMA 6.1.2.5 /02	Seehase, S.	2016	LITERATURE REVIEW ON BACILLUS THURINGIENSIS SUBSP. KURSTAKI SA-12: TOXICOLOGY Certis USA LLC GAB Consulting GmbH, Stade, Germany Report-no.: 2281384-MA-05-01_SA-12 GLP/GEP: no Published: no	no	yes	Protected	Certis USA	New data for active ingredient, not previously submitted nor evaluated

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KMA 6.1.2.5 /03	Barfod, K.K., Poulsen, S.S., Hammer, M., Larsen, S.T.	2010	SUB-CHRONIC LUNG INFLAMMATION AFTER AIRWAY EXPOSURES TO BACILLUS THURINGIENSIS BIOPESTICIDES IN MICE BMC Microbiology, 3, 10:233 Report-no.: not applicable GLP/GEP: no Published: yes	yes	no	not protected	-	New data for active ingredient, not previously submitted nor evaluated