Renewal Assessment Report

Bacillus thuringiensis subsp. *aizawai strain GC-91*

Volume 3 – B.6 Effects on human health

July 2018

Rapporteur Member State: The Netherlands

Co-Rapporteur Member State: Germany

Version history

When	What
July 2018	Initial RAR

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B Summary of the data and information

B.6 Effects on human health

Bacillus thuringiensis subsp. aizawai GC-91 (in the following abbreviated as Bta GC-91) was included in Annex I of Council Directive 91/414/EEC in 2008. Subsequently Regulation (EC) No 1107/2009 repealed and replaced Directive 91/414/EEC and the active substance Bta GC-91 was deemed to be approved under that Regulation and included in the Annex to Regulation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. aizawai* (strains ABTS-1857, GC-91) on the 19 December 2012 (published January 2013). Based on this new information available, no need to change the conditions of approval of Bta GC-91 was identified. The Commission filed on 13 December 2013 an updated review report for Bta GC-91 to the Standing Committee on the Food Chain and Animal Health for examination.

The approval of Bta GC-91 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 Bta GC-91 on 30 April 2016.

Bta GC-91 is a transconjugant strain originating from a Bta and a Bt subsp. kurstaki strain. Bta in general occurs ubiquitous in soils on plants as well as in infested insects. Bta 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 Bta is mainly attributed to spore bound insecticidal pro-toxins (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 (2013) confirms that no critical areas of concern are identified within the framework of the use which was supported.
As the manufacturing process of Bta GC-91 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.

Besides new information, the submitted dossier includes all data, which have been presented in the DAR (Jan 2008) and DAR addendum (Nov 2012). This information is marked grey with a clear indication where the information is originating from.

B.6.1 Tier I

B.6.1.1 Basic information

Information from DAR (2012) *Bacillus thuringiensis* has been used in agriculture for many years to reduce damage from several species of insect larvae, primarily of the order Lepidoptera, in a variety of food, feed and fiber crops. More recently other *B. thuringiensis* isolates have been shown to be effective in controlling insect larvae of two additional orders: *Diptera* and *Coleoptera.* Many subspecies of *B. thuringiensis* have been economically produced on a commercial scale all over the world since 1965. Since that time many hundreds of thousands of metric tons of *B. thuringiensis* have been used for crop protection on a world-wide basis. Throughout this period *B. thuringiensis* has proven not only to be an effective larval control agent but also to pose no hazard to human health and to be environmentally safe, especially to mammals, birds and aquatic ecosystems (Siegel 2001). Because of their environmental safety the *B. thuringiensis* products are considered excellent biological insecticides for use in agriculture or for other applications such as forestry and aquatic systems.

The subspecies *aizawai* and *kurstaki* only differ in their specificity and their efficacy to lepidopteran species. All the other physiological, biochemical and biological properties are similar. Therefore, in this dossier, data on other subspecies and strains will be consulted to support the results of *Bacillus thuringiensis* subspecies *aizawai* strain GC-91 or to make data available for extrapolation.

In order to support the Annex I inclusion of the active substance *Bacillus thuringiensis aizawai*, the company Mitsui submits data for *B. thuringiensis aizawai* strain GC-91, the active ingredient of the biological plant protection product Agree 50 WP.

New data 2016

Relevant information has already been submitted for first approval of Bta GC-91 and is still considered acceptable to cover current requirements. The review report for Bta GC-91 indicated no harmful effects on human or animal health by Bta GC-91.

A literature search according to EFSA (2011) was conducted in May 2016 covering the last 10 years. The literature research was carried out and 13 references were considered relevant and reliable and are summarised under the respective data points below. For more details please refer to Seehase (2016) and B.6.4. Data requirements for metabolites of Bta are covered by the literature search. Btas´ and Btks´ insecticidal proteins were shown to be of no concern for human health. Please refer to B.2.8. Additionally, the close relationship and the toxigenic potential of Bta due to possible production of *B. cereus* enterotoxins is in detail discussed in B.2.8.

Although Bta/Btk is capable of producing diarrhoeal enterotoxins no diarrhoea or serious health issues have been observed in rats with human gut flora at high dose levels of Btk (Wilcks et al. 2006). 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 did not increase significantly over a 3-year period (Baelum et al., 2012).

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

B.6.1.1.1 Medical data

Information from DAR (2012) No detailed medical data about *Bacillus thuringiensis aizawai* medical surveillance were submitted.

No data were published or reported about possible infection or pathogenicity on humans due to *Bacillus thuringiensis* subsp. *aizawai*.

New data 2016

From the recent literature search, no references were identified, reporting medical cases of Bta GC-91 or Bta in general. For more details please refer to Seehase (2016, see B.6.4).

B.6.1.1.2 Medical surveillance on manufacturing plant personnel

Information from DAR (2012) Volume 3 Point B.6.1.1.2/ OECD Dossier Doc M-IIM, Section 3, Point IIM 5.2.1

In the medical records provided by Mitsui no adverse health effects attributable to *Bacillus thuringiensis* exposure were found in physical examinations in workers exposed to *Bacillus thuringiensis* during production, filling and packaging. Exposure levels were determined in different areas of the manufacturing process (estimated worst case 0.51 – 12.5 × 10^9 spores), but no specific examination were performed at the study time (1984 - 1987).

New data 2016

A new health surveillance report is submitted for renewal of Bta GC-91 under Regulation (EC) No 1107/2009 by the sponsor. No incidents related to adverse health effects to employees, resulting from exposure to Bta GC-91 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 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 mammals.

Cited references:

Reference	**KMA 5.1.2/01**
Report	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. 2012;19(4), pp. 631-636
Guideline:	Not applicable
GLP:	No

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 harzenianum* 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. harzenianum*, 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.

B.6.1.1.3 Sensitisation/allergenicity observations, if appropriate

Information from DAR (2012) Volume 3 Point B.6.1.1.3/ OECD Dossier Doc M-IIM, Section 3, Point IIM 5.2.2.

Bacillus thuringiensis is classified as sensitising by the EU authorities on the basis of the animal study results.

Exposure to microbial biopesticides based on *Bacillus thuringiensis* may stimulate the production of specific humoral IgE-and IgG antibodies, as showed by immunoassay or ELISA analysis in samples of sprayed workers.

Bacillus thuringiensis kurstaki has been detected at low levels in nasal swaps and as vegetative form in faeces of workers and residents exposed to *Bacillus thuringiensis*. While an increase in humoral antibodies (mainly IgE and IgG) towards *Bacillus thuringiensis* was detected upon occupational exposure, no statistically clinical adverse health effects were noted. Nearly all the workers exposed to higher concentrations for several shifts (5 to 20) were culture-positive for *Bacillus thuringiensis*, and the majority of the workers remained culture-positive for 14 to 30 days. Of those who were culture-positive, eight workers reverted to a culture-negative status during the project or within 30 days of project completion. During the spray programme, some workers experienced chapped lips, dry skin, eye irritation, and nasal drip and stuffiness. These symptoms were transient and frequently occurred during the beginning of a spray run and when *Bacillus thuringiensis* spray concentrations were increased (Bernstein 1999).

In a cohort of Danish greenhouse workers exposed to commercial products, induction of specific IgE antibodies against *Bacillus thuringiensis* was found, during a three years follow-up Doekes 2004).

In another study no increase in clinical asthma symptoms following aerial exposure to *Bacillus thuringiensis* was recorded in children with asthma.

These study results suggest that the exposure to *Bacillus thuringiensis* sprays may confer a risk of IgE-mediated sensitization, in workers and with less extent bystander

New data 2016

Data provided for first approval are considered acceptable to cover current requirements. Apart from results of a literature search covering the last 10 years, no substantial new data are submitted.

In the literature search covering the last 10 years and focussing toxicity or pathogenicity of Bta and 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 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, 1100000 CFU/m³, 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 *B. thuringiensis*, 53% of the samples had IgE values above the detection limit; however, the majority of the persons was exposed to more than one type of biopesticide. Interestingly, despite increased IgE values to *Bacillus thuringiensis* 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.

Cited references:

Reference	**KMA 5.1.3/01**
Report	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
Guideline	Not applicable
GLP	No

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.

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Reference	**KMA 5.1.3/02 (=KMA 5.1.2/01, see B.6.1.1.2)**
Report	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. 2012;19(4), pp. 631-636

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

Information from DAR (2012) Volume 3 Point B.6.1.1.4/ OECD Dossier Doc M-IIM, Section 3, Point IIM 5.2.3 and Point IIM 5.2.4.

Bacillus thuringiensis is capable of producing diarrhoeal enterotoxins at a level one order of magnitude lower as compared to *Bacillus cereus*, but the significance of which as a cause of human disease is at the moment not known.

Bacillus thuringiensis has been isolated in only a few cases of human bacterial infection, which were mainly related to immunosuppressed condition of the patients made them susceptible to infection. However, these findings poses the question if *Bacillus thuringiensis* may have potential to causing disease in immunocompromised persons.

Experimental data on immunosuppressed (athymic or drug induced) laboratory animals, demonstrated that the kinetics of clearance differ between athymic and euthymic mice as well as between corticosteroid-treated and untreated euthymic mice. Mice immunosuppressed by intravenous injection of cyclophosphamide (200 mg/kg bw) were infected by an application of a bacterial suspension containing, respectively, 10^5, 10^6, or 10^7 CFU per mouse of *Bacillus thuringiensis konkuzian* (serotype H34). Lesions healed spontaneously after 48 h in non-immunosuppressed mice but increased in the other group. Two days after the infection, the mice were sacrificed, *and Bacillus thuringiensis* was recovered only in the samples from the immunosuppressed mice. The bacterial count was correlated with the quantity of the cutaneous bacterial inoculum. Bacteriological examination revealed that *Bacillus thuringiensis* was associated with tissue necrosis and polymorphonuclear infiltrates (Hernandez 1998)

Based on animal experimental data and human clinical findings, *Bacillus thuringiensis* infection could occur in human only when several conditions were satisfied: a severe tissue injury with a massive necrosis, a primary or a secondary immunosuppression and a relevant inoculum at the site of the infection (Hernandez 1999 and 2000; .

Since these condition are exceptional, immunosuppressed individuals do not face any known increased risk of infection using products based on *Bacillus thuringiensis* in the normal condition of use including PPE measures.

New data 2016

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

B.6.1.2 Basic studies

B.6.1.2.1 Sensitisation

Information from DAR (2012) *Bacillus thuringiensis* is classified as a sensitizer by the EU authorities.

New data 2016

No new study is submitted for renewal of the approval of Bta GC-91 under Regulation (EC) No 1107/2009.

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 Bta GC-91 under Regulation (EC) No 1107/2009. According to Regulation (EC) 283/2013 (footnote 1 to point 5.2.1 in Part B) micro-organisms are considered potential sensitizers and a corresponding warning phrase is required. However, the applicant **does not** recommend to **use the warning phrase "Contains *B. thuringiensis* subsp. *aizawai*. Micro-organisms may have the potential to provoke sensitising reactions"** for the following reasons:

- For microorganisms currently approved in the EU, positive reports on sensitisation are absent for bacterial species
- As there are no appropriate test methods, it is impossible to demonstrate absence of sensitisation potential and evaluators therefore strongly rely on published literature, where very little reports on sensitisation caused by species used for plant protection are found. Reports on sensitisation caused by microbials are mostly restricted to moulds, often in combination with moisture in buildings. On the other hand, non-pathogenic bacteria are considered to be able to protect human from sensitisation. This is also confirmed by the EFSA External report "Literature search and data collection on RA for human health for MO used as PPP" (Hackl et al. 2015)[1].

[1] Evelyn Hackl, Margit Pacher-Zavisin, Laura Sedman, Stefan Arthaber, Ulla Bernkopf, Günter Brader, Markus Gorfer, Birgit Mitter, Aspasia Mitropoulou, Monika Schmoll, Willem van Hoesel, Elisabeth Wischnitzky, and Angela Sessitsch, 2015. Literature search and data collection on RA for human health for microorganisms used as plant protection products Reference. EFSA supporting publication 2015:EN-801. 173 pp.

- If exposure to microorganisms during use of plant protection products is compared to "natural" exposure in home or outdoor environments, plant protection products will hardly and only in rare cases exceed natural exposure.
- In other regulatory areas, microorganisms are not considered as potentially sensitising by default although exposure may considerably exceed the one in plant protection. Again sensitisation is restricted to fungi, whereas bacteria and yeasts are considered to be beneficial with respect to human health (Martel et al., 2010).[2]

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, which were, however, only qualitatively measured and therefore not correlated to exposure. Moreover, the prevalence rate ratios among exposed increased only marginally over a relatively long observation period of 3-years (Baelum et al, 2012). For more information, please refer to B.6.1.1.2 above.

Reply RMS
In the EU the default assumption of potential sensitisation for all micro-organisms is currently under discussion. There are indications that certain groups of micro-organisms (e.g. bacteria) are not sensitising, however, as the discussion in ongoing and no consensus is reached yet, the RMS proposes to use the warning phrase **"Contains *B. thuringiensis* subsp. *aizawai*. Micro-organisms may have the potential to provoke sensitising reactions".**

However, based on the available information, the default assignment of PPE such as gloves and respiratory equipment should be carefully considered. As the product is a nearly dust-free granule (see Volume 3 B.2; Dust content before storage "nearly dust-free (2.99 mg) and after storage: nearly dust-free (3.71 mg), the respiratory exposure is negligible during mixing and loading, and respiratory equipment is therefore not considered necessary. For skin sensitisation, penetration through the skin is a prerequisite, as systemic exposure is needed to trigger the immune system. Micro-organisms are considered too large to penetrate the skin. Therefore, the assignment of gloves also seems to be redundant. Moreover, it should be kept in mind that there is no proof that Bta GC-91 is a sensitiser, it is only a default assumption that micro-organisms could be potential sensitisers. The RMS is therefore of the opinion that for Bta GC-91 no PPE should be assigned on the basis of the hazard characteristics. However, in the end the final conclusion on the need for PPE is up to Member States at the time of product authorization/renewal.

B.6.1.2.2 Acute toxicity, pathogenicity and infectiveness

Acute oral toxicity, pathogenicity and infectiveness

Information from DAR (2012) CGA-237218 Technical was mixed with deionised water to produce a 25% (w/v) concentration. Five rats of either sex were given a single dose of test material, by gavage at a dose of 5050 mg per kg b.w. in a volume of 20.2 mL per kg bw corresponding to 1.1×10^{10} CFU per kg b.w. One male died during the study, clinical signs for this male rat included nasal discharge, salivation and upon necropsy the gastrointestinal tract was found to be distended with gas which may be treatment related. No further mortalities were observed. No treatment-related clinical signs of toxicity were observed.

The acute oral LD_{50} of CGA-237218 Technical FL910331was greater than 5050 mg per kg bw.

The preparation does not warrant classification as being toxic or harmful on the basis of its acute oral toxicity studies.

Acute CGA-237218 technical was suspended in deionised water and fifteen rats of either sex were given a single dose of test material, by gavage at a dose of 9.4×10^8 CFU/kg b.w. No mortalities were observed. Clinical signs of toxicity were not observed. The body weight gain of the treated animals was similar to that of untreated animals. The gross necropsy conducted at termination of the study revealed no observable abnormalities. No viable organisms were found in body organs, blood or urine. No signs of infectivity were noted and bacteria were cleared from the animals within 21 days.

The acute oral LD_{50} of CGA-237218 technical was greater than 9.4×10^8 CFU per kg bw.

[2] Martel, Cyril; Nielsen, Gunnar D.; Mari, Adriano; Licht, Tine Rask; Poulsen, Lars Kærgaard. 2010. Bibliographic review on the potential of microorganisms, microbial products and enzymes to induce respiratory sensitization. EFSA supporting publication 2010 Volume 7, Issue 9, 95pp

The preparation does not warrant classification as being toxic or harmful on the basis of its acute oral toxicity studies.

Reference KIIM 5.3.2/01

Report [redacted] (1991)

CGA-237218 Technical FL910331: Acute oral toxicity in rats with a microbial Pest Control Agent (MPCA)

Unpublished Report No 8375-91

Guideline US EPA 81-1

GLP Yes

Materials and Methods: The study was conducted during the period 05.09.-19.09.1991 by [redacted]

CGA-237218 Technical was mixed with deionised water to produce a 25% (w/v) concentration. Five rats of either sex were given a single dose of test material, by gavage at a dose of 5050 mg per kg b.w. in a volume of 20.2 mL per kg bw corresponding to 1.1×10^{10} CFU per kg b.w.

Animals were observed for mortality and clinical/behavioural signs of toxicity three times on the day of dosing and once daily thereafter for 14 days. Individual body weights were recorded prior to dosing and on days 7 and 14.

Findings: One male died during the study, clinical signs for this male rat included nasal discharge, salivation and upon necropsy the gastrointestinal tract was found to be distended with gas which may be treatment related.

No further mortalities were observed.

No treatment-related clinical signs of toxicity were observed. The body weight gain of the treated animals was similar to that expected from untreated animals. The gross necropsy conducted at termination of the study revealed no observable abnormalities.

Conclusions: The acute oral LD_{50} of CGA-237218 Technical FL910331was greater than 5050 mg per kg bw. The preparation does not warrant classification as being toxic or harmful on the basis of its acute oral toxicity studies.

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Reference KIIM 5.3.2/02

Report [redacted] (1990a)

Acute oral toxicity and infectivity/pathogenicity study of CGA-237218 technical (*Bacillus thur.* var. *aizawai*) in rats,

Unpublished Report No 90341D/CBG 517-1/AC

Guideline US EPA FIFRA 154A-10

GLP Yes

Materials and Methods: The study was conducted during the period 30.01. – 20.02.1990 by [redacted]

CGA-237218 technical was suspended in deionised water and fifteen rats of either sex were given a single dose of test material, by gavage at a dose of 9.4×10^8 CFU/kg b.w.

Animals were observed for mortality and clinical/behavioural signs of toxicity frequently times on the day of dosing (day 1) and once daily thereafter for 21 days. Individual body weights were recorded prior to dosing and on days 2, 4, 8, 15, and 22 or at time of death.

Urine and faeces were collected on days 2, 3, 4, and 22 and analysed for *Bacillus thuringiensis*.

Upon necropsy blood, brain, lungs, liver, spleen, kidneys, mesenteric lymph nodes and samples of content of stomach, small intestine and caecum were taken and analysed for *Bacillus thuringiensis*.

Group	Treatment	n	Sacrifice on day (males/females)				
		Males/females	Day 2	Day 4	Day 8	Day 15	Day 22
1	None	2/2	-	-	-	-	2/2

2	None, separate room	2/2	-	-	-	-	2/2
3	Autoclaved CGA-237218 technical	4/4	-	-	-	-	4/4
4	CGA-237218 technical	15/15	3/3	3/3	3/3	3/3	3/3

Findings: No mortalities were observed. Clinical signs of toxicity were not observed.

The body weight gain of the treated animals was similar to that of untreated animals. Body temperatures were unaffected. The gross necropsy conducted at termination of the study revealed no observable abnormalities. No viable organisms were found in body organs, blood or urine. Levels of *Bacillus thuringiensis* in intestinal contents or faeces were high initially but declined until there were none detectable at 21 day post dosing.

Details of the recovery in intestinal tract, faeces, and urine were as follows:

Group (n=6)	**Time after dosing**	**Range of colony forming units per gram/ml**					
		Stomach	**Small intestine 1st loop**	**Small intestine 7th loop**	**Caecum**	**Faeces**	**Urine**
A	24 h	$9.4*10^1$-$7.73*10^4$	LOQ-$6.89*10^2$	$9.11*10^2$-$2.55*10^6$	$1.48*10^4$-$1.58*10^6$	$8.05*10^6$-$5.35*10^7$	LOQ-$1.23*10^2$
	2 d	nd	nd	nd	nd	$8.91*10^5$-$3840000*10^2$	LOQ
B	3 d	LOQ-$5.28*10^2$	LOQ-$1.7*10^1$	LOQ-$1.15*10^2$	LOQ-$4.87*10^3$	$3.07*10^4$-$2.76*10^5$	LOQ
C	7 d	LOQ-$6.7*10^1$	LOQ	LOQ-$2.11*10^2$	LOQ-$7.8*10^1$	nd	LOQ
D	14 d	LOQ	LOQ	LOQ-$6.84*10^2$	LOQ-$2.22*10^2$	nd	LOQ
E	21 d	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
G (n=4)	untreated shelf control	LOQ	LOQ	LOQ	LOQ	nd	nd

LOQ=10 CFU/gram/ml

Conclusions: The acute oral LD_{50} of CGA-237218 technical was greater than 9.4×10^8 CFU per kg bw. No signs of infectivity were noted and bacteria were cleared from the animals within 21 days. The preparation does not warrant classification as being toxic or harmful on the basis of its acute oral toxicity studies.

New data 2016

Data provided for first approval are considered acceptable to cover current requirements. Apart from results of a literature search, no substantial new data are submitted for renewal of Bta GC-91 according to Regulation (EC) No 1107/2009.

In a literature search covering the last 10 years and focussing on toxicity or pathogenicity of Bta and Btk to humans and mammals, one article was identified reporting on oral toxicity. A study by Wilcks et al. (2006) 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.

Thus, there is no evidence that Bta GC-91 may cause acute oral toxicity, pathogenicity or infectivity in mammals.

Cited references:

Reference	**KMA 5.2.2.1/01 (=KMA 2.8/07)**
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 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 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 fed spores, 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. Germination of spores in the intestines was observed in groups fed spores of either strain, and revealed that up to 90% of the cells found in the small gastrointestinal tract (duodenum and ileum) were present as vegetative cells, i.e. germinated spores. 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

Although germination of spores was detected and it is known that both of the investigated *B. thuringiensis* strains produce enterotoxins in vitro, no in vivo production of enterotoxins was detected by application of Vero cell assays to intestinal samples from animals fed with either of the strains.Heat-treatment of *B. thuringiensis* spores prior to dosing, mimicking heating food containing Bt spores, did not affect their activity in the gastrointestinal tract of human-flora-associated rats, however, no health issues associated with *Btk* or *Bti* have been revealed, except one single animals showing *Btk* in the spleen.

Acute inhalation toxicity, pathogenicity and infectiveness

Information from DAR (2012) In an acute inhalation toxicity study, SD rats were exposed to CGA-237218 technical FL-911722 as an aerosol for four hours. Two experiments were conducted each with five rats per sex and exposure levels of 0.526 mg/L and 3.16 mg/L corresponding to 5.6 and 37.7 × 10^6 CFU *Bacillus thuringiensis aizawai* /L. Animals then were observed for 14 days for clinical signs of systemic toxicity. No mortalities were observed. Clinical signs of toxicity observed were transient and slight or very slight and included piloerection, activity decrease and nasal discharge at the lower concentration. In addition, at the higher exposure level lacrimation and ptosis were noted. The gross necropsy conducted at termination of the study revealed no observable abnormalities. Following inhalative exposure of rats to CGA-237218 technical at a dose level of 3.16 mg/L corresponding to 3.77 × 10^7 CFU/kg b.w. no mortalities occurred. Only slight transient signs of toxicity were observed and recovery was complete within 2 days after dosing.

The preparation does not warrant classification as being toxic or harmful on the basis of this inhalative toxicity study.

An intratracheal toxicity, infectivity and pathogenicity study was also described in the original (revised) DAR. Intratracheal instillation at a dose of 1.2 mL per kg bw corresponding to 3.76 x 10^8 CFU/kg b.w. resulted in the death of 1 male and 1 female. Initially transient signs of toxicity were observed in all animals treated with autoclaved and viable Bta GC-91 and recovery was complete within 3 days after dosing. No signs of infectivity were noted and bacteria were cleared from the animals within 21 days, except for two of six animals where amounts of 10^5 CFU per pair of lungs were still recovered from the lungs.

Reference KIIM 5.3.3/02

Report [redacted] (1992)

CGA-237218 technical FL-911722: Acute Inhalation Toxicity Study in Rats with a microbial Pest Control Agent (MPCA)

Unpublished Report No 8374-91

Guideline US EPA 81-3

GLP Yes

Materials and Methods: The study was conducted during the period 04.10. – 30.10.1991 by [redacted]

In an acute inhalation toxicity study, SD rats were exposed to CGA-237218 technical FL-911722 as an aerosol for four hours. Two experiments were conducted each with five rats per sex and exposure levels of 0.526 mg/L and 3.16 mg/L corresponding to 5.6 and 37.7 × 10^6 CFU *Bacillus thuringiensis aizawai* /L. Animals then were observed for 14 days for clinical signs of systemic toxicity.

Exposure parameters	Experiment 1	Experiment 2
Nominal concentration	10.8 mg/L	87.4 mg/L
Actual concentration	0.526 mg/L	3.16 mg/L
	5.6 × 10^6 CFU	37.7 × 10^6 CFU
Mean rel. Humidity	100%	60%
Mass median aerodynamic diameter	< 0.4 µm	11.93 µm
Geometric standard deviation	Not able to calculate	3.91 µm
Proportion of particles < 1.1 µm	76.5%	3.76%
Duration of exposure	4 h	4 h

Findings: No mortalities were observed. Clinical signs of toxicity observed were transient and slight or very slight and included piloerection, activity decrease and nasal discharge at the lower concentration. In addition, at the higher exposure level lacrimation and ptosis were noted. However, animals were without clinical signs by day 2.

The body weight gain of the treated animals was similar to that of untreated animals. The gross necropsy conducted at termination of the study revealed no observable abnormalities.

Conclusions: Following inhalative exposure of rats to CGA-237218 technical at a dose level of 3.16 mg/L corresponding to 3.77 × 10^7 CFU/kg b.w. no mortalities occurred. Only slight transient signs of toxicity were observed and recovery was complete within 2 days after dosing. The preparation does not warrant classification as being toxic or harmful on the basis of this inhalative toxicity study.

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Reference KIIM 5.3.3/01:

Report [redacted] (1990b)

Acute pulmonary toxicity and infectivity/pathogenicity study of CGA-237218 technical (*Bacillus thuringiensis* var. *aizawai*) in rats

Unpublished Report No 90323D/CBG 517-2/AC

Guideline US EPA FIFRA 152A-12

GLP Yes

Materials and Methods: The study was conducted during the period 07.02. – 28.02.1990 by [redacted]

CGA-237218 technical was ball milled to reduce particle size and suspended in physiological saline and eight-

een rats of either sex were given a single dose of test material, by intratracheal instillation at a dose of 1.2 mL per kg bw corresponding to 3.76×10^8 CFU/kg b.w.

Animals were observed for mortality and clinical/behavioural signs of toxicity frequently times on the day of dosing (day 1) and once daily thereafter for 21 days. Individual body weights were recorded prior to dosing and on days 2, 4, 8, 15, and 22 or at time of death.

Urine and faeces were collected on days 2, 3, 4 and 22 and analysed for *Bacillus thuringiensis*.

Upon necropsy blood, brain, lungs, liver, spleen, kidneys, mesenteric lymph nodes and samples of content of stomach, small intestine and caecum were taken and analysed for *Bacillus thuringiensis*.

Group	Treatment	n	Sacrifice on day (males/females)					
		Males/females	Day 1*	Day 2	Day 4	Day 8	Day 15	Day 22
1	None	2/2	-	-	-	-	-	2/2
2	None, separate room	2/2	-	-	-	-	-	2/2
3	Autoclaved CGA-237218 technical	4/4	-	-	-	-	-	4/4
4	CGA-237218 technical	18/18	3/3	3/3	3/3	3/3	3/3	3/3

*1h after dosing

Findings: One male and one female rat died within 7 h after dosing. No further mortalities were observed. Small body weight losses were recorded for both animals that were found dead and autopsy revealed slight haemorrhaging of the lungs in both rats.

Clinical signs of toxicity were observed including piloerection, lethargy and increased salivation which were noted in animals treated with autoclaved or viable *Bacillus thuringiensis*. In addition, some animals showed hunched posture, pallor of extremities, gasping, rales and hyperthermia. However, recovery was complete by day 3 and no further signs of toxicity were observed.

The body weight gain of the treated animals was similar to that of untreated animals. No pyrogenic response was noted. The gross necropsy conducted at termination of the study revealed no observable abnormalities.

No viable organisms were found in body organs, blood or urine except incidental findings in brain, lymph nodes, spleen, urine and blood during the first week. Levels of *Bacillus thuringiensis* in faeces were high initially but none detectable at 21 day post dosing. Initially, high levels of *Bacillus thuringiensis* were recovered from the lungs but after 14 days and 21 days elevated levels were found in only one or two of six animals.

Details of the recovery in organs, blood and excreta were as follows:

Recovery of Bacillus thuringiensis from rat lung following intratracheal dosing

Group	Time after dosing	Rat no.	Colony forming units per pair lungs
A	1 hr	101♂	5.60×10^4
		102♂	1.29×10^5
		103♂	2.05×10^5
		127♀	2.43×10^4
		128♀	1.19×10^7
		129♀	1.08×10^4
B	24 hrs	104♂	<10
		105♂	7.00×10^5
		106♂	RD
		130♀	8.70×10^5
		131♀	13
		132♀	27
C	3 days	107♂	6.75×10^5
		108♂	TNTC
		109♂	83
		133♀	<10
		134♀	<10
		135♀	4.65×10^5
D	7 days	110♂	9.37×10^5
		111♂	<10
		112♂	<10
		136♀	2.00×10^2
		137♀	1.07×10^6
		138♀	RD
E	14 days	113♂	<10
		114♂	5.70×10^5
		115♂	<10
		139♀	<10
		140♀	<10
		141♀	<10
F	21 days	116♂	<10
		117♂	<10
		118♂	3.82×10^5
		142♀	<10
		143♀	2.42×10^5
		144♀	<10
H	Undosed shelf controls	123♂	<10
		124♂	<10
		149♀	<10
		150♀	<10

RD Rat died prior to sacrifice
TNTC Colonies to numerous to count on plates

Recovery of Bacillus thuringiensis from blood and tissues

Group	Time after dosing	Rat no.	Viable counts, colony forming units per g					
			Brain	Liver	Kidney	Spleen	Mesenteric lymph nodes	Blood
A	1 hr	101♂	26	<10	<10	<10	<10	<10
		102♂	63	<10	30	<10	1.11×10^2	5.93×10^2
		103♂	47	<10	<10	<10	<10	1.73×10^2
		127♀	<10	<10	<10	<10	<10	<10
		128♀	1.73×10^3	<10	29	<10	<10	2.86×10^2
		129♀	95	<10	<10	<10	1.11×10^2	<10
B	24 hrs	104♂	<10	<10	<10	<10	<10	<10
		105♂	<10	11	<10	<10	<10	9.68×10^2
		106♂	RD	RD	RD	RD	RD	RD
		130♀	<10	<10	<10	28	<10	<10
		131♀	<10	<10	60	<10	17	<10
		132♀	<10	<10	43	1.55×10^2	<10	<10
C	3 days	107♂	<10	37	61	83	<10	<10
		108♂	38	29	20	4.18×10^2	<10	<10
		109♂	<10	<10	<10	70	<10	<10
		133♀	<10	<10	<10	<10	<10	<10
		134♀	<10	<10	<10	<10	<10	<10
		135♀	<10	<10	<10	1.33×10^2	<10	<10
D	7 days	110♂	<10	<10	<10	22	<10	<10
		111♂	18	13	<10	<10	<10	<10
		112♂	<10	<10	<10	<10	<10	<10
		136♀	<10	<10	<10	<10	<10	<10
		137♀	<10	1.88×10^2	<10	2.51×10^3	<10	<10
		138♀	RD	RD	RD	RD	RD	RD
E	14 days	113♂	<10	<10	<10	<10	<10	<10
		114♂	<10	<10	<10	62	<10	<10
		115♂	<10	<10	<10	<10	<10	<10
		139♀	<10	<10	<10	<10	<10	<10
		140♀	<10	<10	<10	<10	<10	<10
		141♀	<10	<10	<10	<10	<10	<10
F	21 days	116♂	<10	<10	<10	<10	<10	<10
		117♂	<10	<10	<10	<10	<10	<10
		118♂	<10	<10	<10	<10	<10	<10
		142♀	<10	<10	<10	<10	<10	<10
		143♀	<10	<10	<10	<10	<10	<10
		144♀	<10	<10	<10	<10	<10	<10
H	Undosed shelf controls	123♂	<10	<10	<10	<10	<10	<10
		124♂	<10	<10	<10	<10	<10	<10
		149♀	<10	<10	<10	<10	<10	<10
		150♀	<10	<10	<10	<10	<10	<10

RD Rat died prior to sacrifice

Conclusions: Following intratracheal instillation of CGA-237218 technical at a dose level of 3.76×10^8 CFU/kg b.w., two animals died. Initially transient signs of toxicity were observed and recovery was complete within 3 days after dosing. No signs of infectivity were noted and bacteria were cleared from the animals within 21 days, except for two of six animals where amounts of 10^5 CFU per pair of lungs were still recovered from the lungs. The preparation does not warrant classification as being toxic or harmful on the basis of this intratracheal toxicity studies.

New data 2016

Data provided for first approval are considered acceptable to cover current requirements. Apart from results of a literature search, no new studies are submitted for renewal of Bta GC-91 according to Regulation (EC) No 1107/2009.

In the literature search covering the last 10 years and focussing on toxicity of Btk/Bta on mammals, two articles were identified studying respiratory toxicity of mice following exposure to Btk (Barford 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 Bta may cause acute or subchronic respiratory toxicity, pathogenicity or infectivity in mammals.

As a consequence classification and labelling with regard to acute or repeat dose exposure is not necessary.

Cited references:

Reference	**KMA 5.2.2.2/01 (see KMA 5.2.5.1/01 for chronic exposure)**
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.
	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.

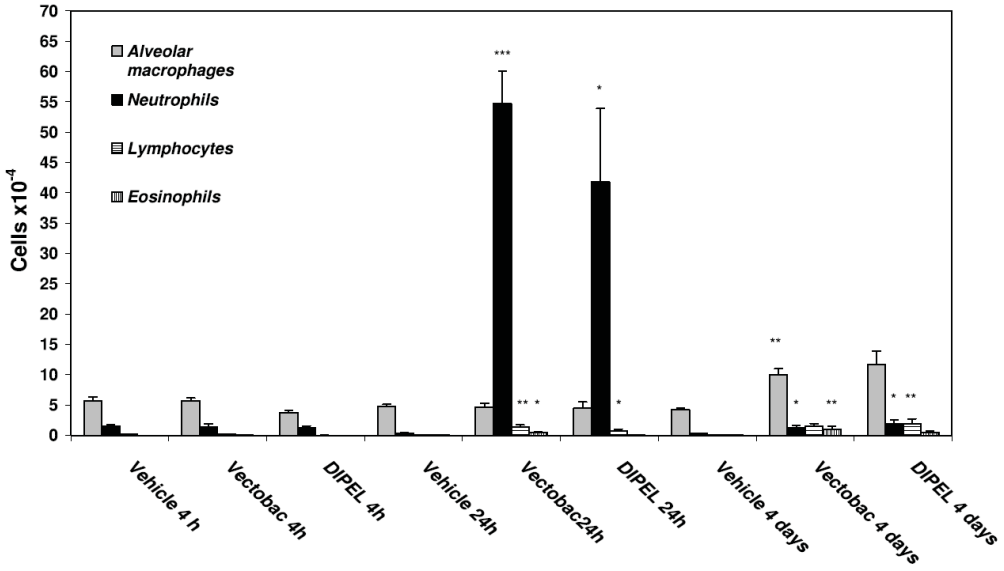

Fig. B.6.1.2.2-1: Cells in bronchoalveolar lavage fluid at different time points after instillation of biopesticides.

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 to the effect.

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Reference	**KMA 5.2.2.2/02**
Report	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, 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 behavioral 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 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.

Intraperitoneal/subcutaneous single dose

Information from DAR (2012) Intravenous

Eighteen male and eighteen female CD rats were injected intravenously each with 3 mL of a ball milled suspension of CGA-237218 Technical at a concentration of 2.53×10^7 CFU/mL. Urine, faeces, caecal contents, blood and organs, including brain, mesenteric lymph nodes, heart, lungs, kidneys, liver and spleen were analysed for viable test organisms. No mortalities and no treatment-related clinical signs of toxicity were observed. No test organisms were recovered from urine and faeces, low numbers were recovered from single animals during the first 24 h from blood, brain, kidney and lymph nodes. Large numbers of viable test organisms were recovered from spleen, liver and lungs of treated rats early in the study. CGA-237218 Technical (*Bacillus thuringiensis* var. *kurstaki/aizawai*) at a dose level of 7.6×10^7 CFU per animal showed no evidence of toxicity or infectivity/pathogenicity to rats following intravenous administration.

Reference	KIIM 5.3.4/04
Report	[redacted] (1990c) Acute intravenous toxicity and infectivity/pathogenicity to rats of CGA-237'218 Technical (*Bacillus thuringiensis* var. *kurstaki/aizawai*), Unpublished Report No. 90324D/CBG 517-3/AC
Guideline:	EPA FIFRA Subdivision M,152A-13
GLP:	Yes

Materials and Methods: The study was conducted during the period 29.01.-19.02.1990 by [redacted]

Eighteen male and eighteen female CD rats were injected intravenously each with 3 mL of a ball milled suspension of CGA-237218 Technical at a concentration of 2.53×10^7 CFU/mL. Animals were observed frequently until sacrifice. Body weight and body temperature were noted. Groups of three rats of either sex were sacrificed at 1 h, 24 h, 3, 7, 14 and 28 days post dosing. Urine, faeces, caecal contents, blood and organs, including brain, mesenteric lymph nodes, heart, lungs, kidneys, liver and spleen were analysed for viable test organisms.

Findings: No mortalities and no treatment-related clinical signs of toxicity were observed. The body weight gain and body temperature of the treated animals was similar to that of control animals. Autopsy findings were normal.

No test organisms were recovered from urine and faeces, low numbers were recovered from single animals during the first 24 h from blood, brain, kidney and lymph nodes. Large numbers of viable test organisms were recovered from spleen, liver and lungs of treated rats early in the study. In the majority of samples the number gradually reduced to non detectable levels by 14 day post treatment. Exception was the spleen where moderate numbers persisted until study termination.

Conclusions: CGA-237218 Technical (*Bacillus thuringiensis* var. *kurstaki/aizawai*) at a dose level of 7.6×10^7 CFU per animal showed no evidence of toxicity or infectivity/pathogenicity to rats following intravenous administration.

Intraperitoneal/subcutaneous single dose

Five male and five female HSD(SMB) mice were each injected intraperitoneously with 0.5 mL of a suspension of CGA-237218 technical 91-7288 corresponding to 1.16×10^6 CFU *Bacillus thuringiensis aizawai* per mouse, respectively. No mortalities and no treatment-related clinical signs of toxicity were observed in any of the mice.

Five male and five female HSD:(SMB) mice were each injected intraperitoneally with 0.5 mL of a suspension of CGA-237218 technical 911445 corresponding to 2.55×10^6 CFU *Bacillus thuringiensis aizawai* per mouse, respectively. No mortalities and no treatment-related clinical signs of toxicity were observed in any of the mice.

Groups of 5 male and 5 female mice were administered an intraperitoneal dose of 1×10^6 , 1×10^7 or 1×10^8 spores of each of five lots of CGA-237218. No mortalities and no treatment-related clinical signs of toxicity were observed in any of the mice at the dose level of 10^6 CFU per animal. A low level of mortalities (10%) occurred at a dose level of 10^7 CFU per animal, while a high frequency of deaths were observed at the highest dose level. Among these, signs of polyuria or diarrhoea and other gastrointestinal effects were noted upon necropsy All mortalities occurred by two days post-treatment. Clinical findings among the two higher dose groups included decreased activity, piloerection, ptosis and ataxia. Nearly all affected animals died. The intraperitoneal LD_{50} for CGA-237218 Technical (*Bacillus thuringiensis* var. *aizawai*) in mice was $> 1 \times 10^7$ CFU per animal. No evidence of toxicity or pathogenicity to mice was observed following intraperitoneal administration at the dose level of 10^6 CFU/animal.

Reference	KIIM 5.3.4/01
Report	[redacted] (1992a) CGA-237218 technical 91-7288: Acute intraperitoneal toxicity/pathogenicity screen in mice Unpublished Report No. 8515-91
Guideline	none
GLP	Yes

Materials and Methods: The study was conducted during the period 31.10. - 07.11.1991 by [redacted] Five male and five female HSD(SMB) mice were each injected intraperitoneously with 0.5 mL of a suspension of CGA-237218 technical 91-7288 corresponding to 1.16×10^6 CFU *Bacillus thuringiensis aizawai* per mouse, respectively. Animals were observed frequently until sacrifice on day 7 post treatment. Body weight was noted before and on day 7 post treatment

Findings: No mortalities and no treatment-related clinical signs of toxicity were observed in any of the mice. The body weight gain was unaffected and autopsy findings were normal.

Conclusion: CGA-237218 Technical 91-7288 (*Bacillus thuringiensis* var. *aizawai*) at a dose level of 1.16×10^6 CFU per animal showed no evidence of toxicity to mice following intraperitoneal administration.

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Reference	KIIM 5.3.4/02
Report	[redacted] (1992b) CGA-237218 technical 911445: Acute intraperitoneal toxicity/pathogenicity screen in mice Unpublished Report No. 8648-91
Guideline:	none
GLP:	Yes

Materials and Methods: The study was conducted during the period 30.12.1991 - 06.01.1992 by [redacted] Five male and five female HSD:(SMB) mice were each injected intraperitoneally with 0.5 mL of a suspension of CGA-237218 technical 911445 corresponding to 2.55×10^6 CFU *Bacillus thuringiensis aizawai* per mouse, respectively. Animals were observed frequently until sacrifice on day 7 post treatment. Body weight was noted before and on day 7 post treatment

Findings: No mortalities and no treatment-related clinical signs of toxicity were observed in any of the mice. The body weight gain was unaffected and autopsy findings were normal.

Conclusion: CGA-237218 Technical 911445 (*Bacillus thuringiensis* var. *aizawai*) at a dose level of 2.55×10^6 CFU per animal showed no evidence of toxicity to mice following intraperitoneal administration.

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Reference KIIM 5.3.4/03

Report [redacted] (1991)

Acute intraperitoneal toxicity/pathogenicity screening studies of technical CGA-237218 in mice,

Unpublished Report No. CBG 517-3

Guideline EPA FIFRA Subdivision M,151A-12

GLP Yes

Materials and Methods: The study was conducted during the period 09.04.1991 – 01.05.1991 by [redacted] Groups of 5 male and 5 female mice were administered an intraperitoneal dose of 1×10^6 , 1×10^7 or 1×10^8 spores of each of five lots of CGA-237218. Animals were observed frequently until sacrifice on day 7 post treatment. Body weight was noted before and on day 7 post treatment. Gross necropsies were performed on the animals at the time of discovery after death or at termination of the study.

Findings: No mortalities and no treatment-related clinical signs of toxicity were observed in any of the mice at the dose level of 10^6 CFU per animal. A low level of mortalities (10%) occurred at a dose level of 10^7 CFU per animal, while a high frequency of deaths were observed at the highest dose level. All mortalities occurred by two days post-treatment.

Incidences of mortalities following I.p. dosing of CGA-237218 technical

Batch	Approximate dose (CFU/mouse)		
	10^6	10^7	10^8
FL-901966	0/10	0/10	8/10
FL-910039	0/10	1/10	10/10
FL-910040	0/10	4/10	10/10
FL-910041	0/10	0/10	5/10
FL-910042	0/10	0/10	8/10

Number of dead mice/Total Number of treated mice

Clinical findings among the two higher dose groups included decreased activity, piloerection, ptosis and ataxia. Nearly all affected animals died. Among these, signs of polyuria or diarrhoea and other gastrointestinal effects were noted upon necropsy. The body weight gain was unaffected and autopsy findings were normal in surviving animals.

Conclusion: The intraperitoneal LD_{50} for CGA-237218 Technical (*Bacillus thuringiensis* var. *aizawai*) in mice was > 1×10^7 CFU per animal. No evidence of toxicity or pathogenicity to mice was observed following intraperitoneal administration at the dose level of 10^6 CFU/animal.

Acute subcutaneous toxicity

In a subcutaneous injection study, three albino mice per sex received the test material CGA-237218 technical as a 10% (w/v) suspension in sterile saline at a dose of 3.8×10^6 CFU/animal at two injection sites (50 µL each). No mortalities and no signs of systemic toxicity occurred. CGA-237218 technical FL 900815 produced mean irritation grades of 3.3 for both erythema and oedema. No mortalities occurred but CGA-237218 technical FL 900815 proved to be extremely irritating upon subcutaneous application.

In a subcutaneous injection study, three albino mice per sex received the test material CGA-237218 technical as a 10% (w/v) suspension in sterile saline at a dose of 2.66×10^6 CFU/animal at two injection sites (50 µL each). No mortalities and no signs of systemic toxicity occurred. No mortalities occurred and CGA-237218 technical FL 900816 proved to be not irritating upon subcutaneous application

In a subcutaneous injection study, three albino mice per sex received the test material CGA-237218 technical as a 10% (w/v) suspension in sterile saline at a dose of 1.08×10^6 CFU/animal at two injection sites (50 µL each). No mortalities and no signs of systemic toxicity occurred.

Reference KIIM 5.5.1/02

Report [redacted] (1990a)
CGA-237218 technical FL 900815: Mouse subcutaneous injection,
Unpublished Report No 7008-90

Guideline Not mentioned

GLP Yes

Materials and Methods: The study was conducted between 24.04. - 01.05.1990 at [redacted] In a subcutaneous injection study, three albino mice per sex received the test material CGA-237218 technical as a 10% (w/v) suspension in sterile saline at a dose of 3.8×10^6 CFU/animal at two injection sites (50 µL each). Observations for dermal irritation and clinical signs were made at 24, 48 h, 72 h and 7 days after dosing.

Findings: No mortalities and no signs of systemic toxicity occurred. The body weight gains were within the range expected for mice used in this type of study and are therefore considered not indicative of toxicity. CGA-237218 technical FL 900815 produced mean irritation grades of 3.3 for both erythema and oedema.

Conclusion: No mortalities occurred but CGA-237218 technical FL 900815 proved to be extremely irritating upon subcutaneous application.

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Reference KIIM 5.5.1/03

Report [redacted] (1990b)
CGA-237218 technical FL 900816: Mouse subcutaneous injection,
Unpublished Report No 7009-90

Guideline: Not mentioned

GLP: Yes

Materials and Methods: The study was conducted between 24.04. - 01.05.1990 at [redacted] In a subcutaneous injection study, three albino mice per sex received the test material CGA-237218 technical as a 10% (w/v) suspension in sterile saline at a dose of 2.66×10^6 CFU/animal at two injection sites (50 µL each). Observations for dermal irritation and clinical signs were made at 24, 48 h, 72 h and 7 days after dosing.

Findings: No mortalities and no signs of systemic toxicity occurred. The body weight gains were within the range expected for mice used in this type of study and are therefore considered not indicative of toxicity. CGA-237218 technical FL 900816 produced mean irritation grades of 0.1 for both erythema and oedema.

Conclusion: No mortalities occurred and CGA-237218 technical FL 900816 proved to be not irritating upon subcutaneous application.

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Reference KIIM 5.5.1/04

Report [redacted](1990c) CGA-237218 technical FL 900814: Mouse subcutaneous injection,
Unpublished Report No 7007-90

Guideline: Not mentioned

GLP: Yes

Materials and Methods: The study was conducted between 24.04. - 01.05.1990 at [redacted] In a subcutaneous injection study, three albino mice per sex received the test material CGA-237218 technical as a 10% (w/v) suspension in sterile saline at a dose of 1.08×10^6 CFU/animal at two injection sites (50 µL each). Observations for dermal irritation and clinical signs were made at 24, 48 h, 72 h and 7 days after dosing.

Findings: No mortalities and no signs of systemic toxicity occurred. The body weight gains were within the range expected for mice used in this type of study and are therefore considered not indicative of toxicity. CGA-237218 technical FL 900814 produced mean irritation grades of 0.1 for erythema and no oedema were observed.

Conclusion: No mortalities occurred and CGA-237218 technical FL 900814 proved to be not irritating

upon subcutaneous application.

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 Bta GC-91 according to Regulation (EC) No 1107/2009.

From the literature search, not a single reference was identified, reporting on effects of Btk after intraperitoneal or subcutaneous application. Only in the study performed by [redacted] (1990a) extremely irritant effects upon subcutaneous application are found. As there will be no dermal penetration expected the active substance does not need to be classified for irritant effects.

Please refer to the literature search report for detailed information on the search strategy (Seehase 2016).

Thus, there is no evidence that Bta GC-91 acts toxic or pathogenic following intravenous/subcutaneous administration.

B.6.1.2.3 Genotoxicity testing

In vitro studies

Information from DAR (2012) Volume 3 Point B.6.1.2.3/ OECD Dossier Doc M-IIM, Section 3, Point IIM 5.3.5.

1.) The test material, CGA 237218 techn. was tested with and without metabolic activation on strains TA 100, TA 1535, TA 98, TA 1537 of *Salmonella typhimurium* and on strain WP2 uvrA of *Escherichia coli* for the induction of gene mutations (reverse mutations) with doses from 20 to 5000 µg/plate (Hertner (1992))

Findings: No increases in *Salmonella typhimurium* and in *Escherichia coli* revertant colony numbers were observed in any of the tester strains, both with or without microsomal enzymes. This was determined in an initial and a confirmatory assay. For the strain TA98 some cytotoxicity was noted.

Conclusions: CGA 237218 technical is non-mutagenic (1992)

Comments

The description of the test substance is not included in the report; in the summary it is stated that the test material CGA 237218 techn. (?) and its metabolites (?) are not mutagenic.

The study is inadequate for its evaluation.

2.) Using the Ames Salmonella assay, Carlberg et al. (1995) investigated the mutagenic potential of concentrated supernatants of cultures of *Bacillus thuringiensis* strains H1 (HAMB1 252) and serotype H-14 (S-128), of pre-purified thuringiensin and of purified endotoxin preparations. These were tested at dose levels of 0.5 – 50 µL of a 10-fold concentrated supernatant in the Salmonella reverse mutation assay without and with external metabolic activation with rat liver homogenate (S9). The test battery included strains TA98, TA100, TA1535, TA1537 and TA1538 of *Salmonella typhimurium* in two independent plate incorporation tests. No reproducible increases in revertant colony numbers were observed in any of the tester strains with either test item. The preparations are non-mutagenic.

Material tested:

- Preparation I: 10-fold concentrated supernatant culture medium of B. thuringiensis, Serotype H-1 (1.1×10^9 spores/mL; LD_{50} = 2.7µl/ml)
- Preparation II: pre-purified thuringiensin with LD_{50} = 2.3 µL/mL (extracted with ethanol)
- Preparation III: 10-fold concentrated supernatant culture medium of *B.thuringiensis* Serotype H-14 (4.4×10^9 spores/mL; LD_{50} = 0 0.27 µL/mL)
- Preparation IV: purified lyophilized endotoxin preparation with LD_{50} = 2.9 ng/mL (extracted with ethanol).

Results:

Preparation I (supernatant H-1 x 10)	Increase in TA 1538 with no biological significance (less than 2 times)	NOT MUTAGENIC
Preparation II (thuringiensin from H-1)	Increase in TA 98 and TA 1538 with no biological significance (less than 2 times in two exps.)	NOT MUTAGENIC
Preparation III (supernatant H-14)	Increase in TA 98/TA 1538 with no biological significance (less than 2 times in two exps.)	NOT MUTAGENIC
Preparation IV (purified endotoxin)	Negative results in TA 98 and in TA 100	NOT MUTAGENIC

Remarks

Some of the positive results noted by the AA. Could be due to the histidine content of the test material, as stated also by the AA.

No specific information are presented on the chemical nature (f.i. purity) of the tested material.

The study is inadequate for its evaluation

3.) In an *in vitro* study by Meretoja et al. (1977), supernatants from *Bacillus thuringiensis* serotype 1 or serotype 3 were applied to human blood cultures at dose levels of 20% (v/v) of supernatant in the medium. Cytotoxicity was determined as inhibition of mitotic activity. In order to determine the clastogenicity, the number of aneuploid metaphases and the occurrences of structural chromosome and chromatid aberrations were recorded. While cytotoxic concentrations of the exotoxin-producing strain (*Bacillus thuringiensis* serotype 1) caused significantly increased chromosomal aberrations, no significant clastogenic effect was observed with supernatants *Bacillus thuringiensis* serotype 3 (ssp. *kurstaki*). Since *Bt* ssp. *aizawai* is very similar to *Bt* ssp. *kurstaki* and also does not produce any exotoxins, it may be assumed that Bta will also have no clastogenic effect.

Comments

End points analysed:	chromosome aberrations in human lymphocytes
Material tested:	autoclaved supernatant of B. thuringiensis serotype 1 (AS1);
	purified exotoxin received from Shell, UK;
	autoclaved supernatant of Bacillus thuringiensis serotype 3 (AS3) not producing exotoxin
Results	**Chromosome breaks were induced by both supernatants (AS1 and AS3-not producing exotoxin), but not from exotoxin.**

This result is unclear and cannot be accepted without information on the chemical nature of the tested material

The study is inadequate for its evaluation

Genotoxicity testing of microbial preparation is required in the European Union and in the US legislation for registration of plant protection active substances. However, a series of technical and scientific problems related to the nature of the material to be submitted to the testing protocols has not been discussed and solved.

The guidelines currently in place for genotoxicity testing have been developed to test chemicals. The use of these guidelines poses certain problems when microbial testing is envisioned. It is recognized that the physicochemical properties of a substance (e.g., volatility, pH, solubility, stability, its purity, complex mixture, biomaterial property, etc.) can sometimes make standard test conditions inappropriate. This becomes even more apparent as one considers microbial organisms. Standard mutagenicity and genotoxicity assays are not considered appropriate for many living microorganisms nor does the risk they pose often warrant such testing.

EC Directive 91/414 - 5.2.3 Genotoxicity testing

Circumstances in which required

If the micro-organism produces exotoxins according to point 2.8, then these toxins and any other relevant metabolite in the culture medium must also be tested for genotoxicity. Such tests on toxins and metabolites should be performed using the purified chemical if possible.

If basic studies do not indicate that toxic metabolites are formed (acute/chronic toxicity studies?) studies on micro-organism itself should be considered depending on expert judgment on the relevance and validity of the basic data. In the case of a virus the risk of insertional mutagenesis in mammalian cells or the risk of carcinogenicity has to be discussed.

EPA CFR

EPA's data registration for microbial pesticides conditionally requires mammalian mutagenicity data (158.740) at Tier II level when:

- acute infectivity tests are positive in Tier I studies;
- adverse effects are observed in immune response studies;
- positive results are obtained in tissue culture tests with viral agents.

OECD

The OECD Guidance for Testing of Chemicals for the genotoxicity tests gives some advice on the susceptibility of the individual tests to the physical characteristics of the test material and they give some advice on compensatory measures that may be taken. (Low solubility, for example, limits all in-vitro mutagenicity tests recommended.). Yet, the lack of a common understanding of several issues that are considered important for the development of a modern general strategy for genotoxicity testing of microorganisms and the subsequent interpretation of test results for risk assessment pose substantial problems.

General discussion

By evaluating the data submitted for registration at the national levels or at the EU level (revision), many procedures have been applied in the preparation of the material to be tested. Here are some examples for bacterial material:

1. Test article: Preparation I: 10-fold concentrated supernatant culture medium of *B. thuringiensis*, (1.1×10^9 spores/ml; LD_{50} = 2.7µL/mL); Preparation II: pre-purified thuringiensin with LD_{50} = 2.3 µL/mL (extracted with ethanol); Preparation III: 10-fold concentrated supernatant culture medium of *B. thuringiensis* (4.4×10^9 spores/mL; LD_{50} = 0.27 µL/mL); Preparation IV: purified lyophilized endotoxin preparation with LD_{50} = 2.9 ng/mL (extracted with ethanol).

2. Test article: Tan powder: XXXX technical (YYYY) technical concentrate (SDTC) of *Bacillus thuringiensis* subspecies *kurstaki*. Batch 10890: 2.41×10^{10} colony forming units (cfu)/g. For testing, a 10% suspension of the test article was prepared in deionized water (= 0.24×10^{10} cfu/mL). This suspension was then centrifuged at 3200 rpm for 10 min. The supernatant was removed and filtered twice using a 0.45 micron filter. The filtrate was then dilute as necessary with deionised water.

3. Test article: The test article has been tested as an extract: A 10% suspension (100 mg/mL = 0.24×10^{10} cfu/ mL) was prepared in McCoy's 5a culture medium. This suspension was vortexed vigorously until all the test article is suspended; then it was centrifuged for 10 min at 3000 rpm. Only the top opaque liquid layer is removed, and the remaining fraction (liquid brown sludge-like middle layer) was filter sterilised through a syringe using a 0.45 µ and Nagene vacuum type filter. The extractant was kept at room temperature till dosing. Dosing volumes of 80% is used to achieve the concentrations tested in the range finding assays.

4. Test article: a dimethyl sulphoxide extract of *Bacillus thuringiensis* subsp. *aizawai* strain was prepared and was shown to be non-mutagenic in a standard bacterial mutagenicity assay.

In all cases no specific parameters to identify the material submitted to the tests are defined, in order to allow other laboratories to repeat the tests in the same conditions.

In all cases positive control material able to demonstrate the adequacy of the technical procedures employed have never been applied: In the scientific literature are not known studies that demonstrate that submitting the biological material (microbial preparation) to the same procedures reported in the case above indicated, positive mutagenic results have been obtained.

The base set of mutagenicity tests normally applied are inadequate and not applicable for the evaluation of the genotoxic potential of microbial material for the following reasons:

1. The bacterial assay used in genotoxicity testing is a reverse mutation assay, based on bacterial strains that carry a defective mutant gene making the strains unable to synthesize the aminoacid histidine or tryptophan

from the ingredients present in the culture medium. A bacterial fermentation broth contains these aminoacids, thus making the test inapplicable.

2. In the standard test for mammalian cell gene mutation suggested by OECD (and EC) mutant cells are able to proliferate in the presence of 6-TG (6-thioguanine), because mutation causes a deficiency in the enzyme HPRT transferase, whereas normal cells are not able to grow. Certain micro-organisms put into such a system may well catalyse the conversion to the toxic ribophosphorylated derivative, thus producing questionable positive results of the test.

3. In vitro mammalian chromosome aberration test to evaluate the clastogenic potential of a microbial agent may produce result due to many microbial products that may cause induction of mitosis in the lymphocytes by non-specific immunological activation lecitine-like. This would produce a false positive due to the spontaneous mutation rate. (MacGregor J. T.: Genetic toxicity assessment of microbial pesticides, 2005).

4. The range and type of genotoxicity studies routinely conducted for pharmaceuticals are not applicable to biotechnology-derived pharmaceuticals and therefore are not needed. The administration of large quantities of peptides/proteins my yield uninterpretable results (ICH: Preclinical safety evaluation of biotechnology-derived pharmaceuticals: 1998).

5. The procedures to prepare lysate from microbial agents to be tested on conventional genotoxicity test methods are extremely variable and cannot be adequately standardized.

6. There are no cases in the scientific literature which show positive mutagenic results when conventional mutagenicity/genotoxicity studies have been applied to microbial agents, including complete bacterial cells, fungal components (mycelium, conidia, spores, etc).

7. If conventional mutagenicity/genotoxicity studies are developed for microbial agents, the protocol to be applied cannot contain positive and negative controls based on similar biological agents, thus making the test uninterpretable.

8. There are cases in the scientific literature indicating that a well described procedure of extraction from fungal cultures have produced an extract found positive in genotoxicity testing. Similar extraction procedures applied to microbial material for genotoxicity testing might employ as positive control one of these cases present in the scientific literature (see below).

Valerie M. Davies and Michael E. Stack: Mutagenicity of Stemphyltoxin, a metabolite of *Alternaria alternate*. A.E.M.: 1991, 180-182, vol 57:

Nancy P. Leller et al: *Aspergillus nidulans* verA is required for production of the mycotoxin Sterigmatocystin. A.E.M.:1994, 1444-1450, vol.60.

Michael E. Stack and Michael J. Prival: Mutagenicity of Alternaria metabolites Altertoxin I, II, and III. A.E.M.: 1986, 718-722, vol.52.

Bing Zhu and Alan M. Jaffrey: Fusarin C: Isolation and identification of two microsomal metabolites. Chem. Res. Toxicol.: 1993, 97-101, vol.6.

M. Bechmann et al.: Toxicity and mutagenicity of molds of the *Aspergillus glaucus* group. Identification of Physcion and three related anthraquinones as main toxic constituents from Aspergillus chevalier .J.Agr. Food Chem.: 1979, 1342, vol. 27.

Francoise Seigle-Murandi et al.: Production, mutagenicity and immunotoxicity of Gliotoxin. J. Agr. Food Chem.: 1990, 1854-1856, vol. 38.

Fu-Xiung Lu and Alan M. Jeffrey: Isolation, structural identification, and characterization of a mutagen from *Fusarium moniliforme*. Chem. Res. Toxicol.: 1993, 91-96, vol.6.

See also: J.MacGregor, Genetic Toxicity Assessment of Microbioal Pesticides: Needs and Recommended Approaches. Report to: Organisation for Economic Cooperation and Development, Paris, December 9, 2005, pp 17.

New data 2016

No validated methods for genotoxicity testing of microorganisms are available. Moreover, no relevant positive and negative controls exist. Genotoxicity testing should therefore be conducted only for specific metabolites. However, no relevant genotoxic metabolites are known for *Bacillus thuringiensis*. Thus, according to Regulation (EU) No 283/2013, genotoxicity testing is not required and no studies using the Bta GC-91 are submitted.

From the literature search covering the last 10 years and focussing on toxicity of Bta and 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. Exposure to the target tissue (bone marrow) was not demonstrated. Moreover, this study clearly shows that standard assays are not appropriate for testing of mutagenicity and genotoxicity of microorganisms.

Cited literature

Reference	**KMA 5.2.3/01**
Report	Grisolia, C.K., Oliveira-Filho, E.C., Ramos, F.R., Lopes, M.C., Muniz, D.H., Monnerat, R.G. (2009)
	Acute toxicity and cytotoxicity of *Bacillus thuringiensis* and *Bacillus sphaericus* strains on fish and mouse bone marrow
	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 (1975) was performed. Blood smears were prepared, Giemsa stained and 10000 cells per animal were scored.

Findings

No lethality nor effects on cell proliferation in mouse bone marrow by the three strains tested compared to control were detectable. Thus, no cytotoxicity was observed.

Table B.6.1.2.3-1: 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.

B.6.1.2.4 Cell culture study

Information from DAR (2012) Not applicable

New data 2016

Bta GC-91 is not an intracellular replicating micro-organisms. Thus, according to Regulation (EU) No 283/2013, cell culture studies are not required.

B.6.1.2.5 Information on short-term toxicity and pathogenicity

Health effects after repeated oral exposure

Information from DAR (2012) CGA-237218 technical was administered by oral gavage to rats for thirteen weeks at a dose of at least 10^8 CFU per animal per day. Control groups received the vehicle saline or autoclaved test substance. A recovery group was retained for four weeks. Bodyweight, food consumption and clinical observations were recorded, macroscopic necropsy was performed and organ weights were recorded. Viable *Bacillus thuringiensis* were determined from blood and tissue samples and caecal contents. Tissues were histopathologically examined. No treatment-related effect was seen on clinical signs, bodyweight gain, ophthalmoscopy, blood biochemistry, urinalysis, macroscopic or microscopic pathology. Among haematological parameters a possible treatment related reduction in lymphocyte and total white cell count was observed for males receiving active or autoclaved CGA-237218 technical. Counts were seen in treated (4/20) and control animals (2/10), and localised pulmonary inflammation seen in both treated and control rats was not considered treatment related. The study gave no indication of toxicity, infectivity or pathogenicity in the rat following thirteen weeks administration of CGA-237218 technical by oral gavage.

Reference KIIM 5.3.7.1/02

Report [redacted] (1993) CGA-237218 technical: Thirteen week oral toxicity/infectivity in rats, Unpublished Report No. CBG 595/930636

Guideline OECD 408

GLP Yes

Materials and Methods: The study was conducted during the period 20.10.1992 - 17.02.1993 by [redacted].

CGA-237218 technical was administered by oral gavage to rats for thirteen weeks at a dose of at least 10^8 CFU per animal per day. Control groups received the vehicle saline or autoclaved test substance. A recovery group was retained for four weeks.

Bodyweight, food consumption and clinical observations were recorded, blood samples were taken immediately after sacrifice. Macroscopic necropsy was performed and organ weights were recorded. Viable *Bacillus thuringiensis* were determined from blood and tissue samples and caecal contents. Tissues were histopathologically examined.

Findings: No treatment-related effect was seen on clinical signs, bodyweight gain, ophthalmoscopy, blood biochemistry, urinalysis, macroscopic or microscopic pathology.

Among haematological parameters a possible treatment related reduction in lymphocyte and total white cell count was observed for males receiving active or autoclaved CGA-237218 technical.

High counts of *Bacillus thuringiensis* were detected in the caecum but counts were reduced at the end of the 4-week recovery period. A single female showed viable counts in the lungs at the end of the recovery period but this observation was attributed to the gavage dosing procedure.

Counts were seen in treated (4/20) and control animals (2/10), and localised pulmonary inflammation seen in both treated and control rats was not considered treatment related.

Study type	Test item	Dose level	Findings	NOAEL	Report
90 days oral rat	*Bta* CGA-237218 technical	10^8 CFU per animal per day for 13 weeks	No adverse effects	10^8 CFU per animal per day	IIM 5.3.7.1/01 [redacted] (1993)

Conclusions: The study gave no indication of toxicity, infectivity or pathogenicity in the rat following thirteen weeks administration of CGA-237218 technical by oral gavage.

Bacillus thuringiensis was administered in the diet for 5 months to six castrated mixed rambouillet/merino sheep (24 - 34 kg at the beginning of the study) at a dose of 500 mg/kg b.w./day (approximately 10^{12} spores per day).

No treatment-related effect was seen on food consumption, weight gain or clinical chemistry parameters nor were significant gross clinical changes observed. Several blood and tissue samples taken at necropsy were found to be positive for Bt when cultured. Detailed gross and microscopic pathologic examination of the sheep revealed several incidental lesions. However, no treatment related clinically significant findings were observed.

The study gave no indication that *Bacillus thuringiensis* is a pathogen in sheep following oral ingestion of large daily doses for five months (Hadley et al., 1987).

New data 2016

Information provided for first approval is considered acceptable to cover current requirements. 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 5.1/01).

Thus, there is no evidence that Bta GC-91 acts toxic or pathogenic following short-term exposure.

Health effects after repeated inhalatory exposure

Information from DAR (2012) Volume 3 Point B.6.1.2.5.1/ OECD Dossier Doc M-IIM, Section 3, Point IIM 5.2.3

According to a statement by [redacted] no adverse reactions in individuals as a result of contact with *Bacillus thuringiensis aizawai* strain GC-91, during its development, manufacture, preparation or field application have been documented or reported.

New data 2016

Data provided for first approval are considered acceptable to cover current requirements. Apart from results of the literature search on other strains, no substantial new information is submitted for renewal of Bta GC-91 according to Regulation (EC) No 1107/2009.

In the literature search covering the last 10 years and focussing on toxicity or pathogenicity of Bta and Btk to mammals, two articles were 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.

Cited references:

Reference	**KMA 5.2.5.1/01 (see KMA 5.2.2.2/01 for acute exposure)**
Report	Barfod, K.K., Poulsen, S.S., Hammer, M., Larsen, S.T. Sub-chronic lung inflammation after airway exposures to *Bacillus thuringiensis* biopesticides in mice. 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 $1.9 \times$

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 contain 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.

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

Information from DAR (2012) No specific treatment after contact with *Bacillus thuringiensis* is required since *Bacillus thuringiensis* does not cause specific adverse effects on humans and domestic animals. In case of accidental direct contact with *Bacillus thuringiensis* the producer states the below listed "first aid instructions"

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.

New data 2016

As noted for all microorganisms "*Bacillus thuringiensis* subsp. *aizawai* strain GC-91 may have the potential to provoke allergic reactions".

In case of accidental direct contact with *Bacillus thuringiensis* the producer states the above listed “first aid instructions” (Safety Data Sheet, Doc M-MP, Point MP 4.4). Moreover, Bta GC-91 is not multi-resistant to commonly used antibiotics and thus, medical treatment in the rare case of an infection is ensured.

B.6.1.3 Toxicity studies on metabolites and relevant impurities

No further information submitted.

B.6.1.4 Summary and conclusions of Tier I studies

See B.6.3 for the overall summary and conclusion.

B.6.2 Tier II

B.6.2.1 Specific toxicity, pathogenicity and infectiveness studies

Information from DAR (2012)

Acute percutaneous (dermal) toxicity

Reference	KIIM 5.5.1/01
Report	[redacted] (1991a) CGA-237218 technical FL 891267: Acute Dermal Toxicity Study in Rabbits with a Microbial Pest Control Agent (MCPA), Unpublished Report No 7012-90
Guideline	EPA Guideline 152A-11
GLP	Yes

Materials and Methods: The study was conducted during the period 03.05. - 17.05.1990 at [redacted]

In an acute dermal toxicity study, five New Zealand White rabbits per sex were exposed to CGA-237218 technical FL 891267 by the dermal route. Approximately 10% of the body surface was clipped and treated with 2020 mg test substance/kg bw for 24 h. Animals then were observed for 14 days.

Findings: No mortalities and no signs of systemic toxicity occurred at 2020 mg/kg bw, the only dose level tested. Dermal responses are summarised in Table B.6.2.1-1.

Table B.6.2.1-1: Individual skin irritation scores

Animal number	Sex	Day 1		Day 2		Day 4		Day 7		Day 9		Day 14	
		Eryth.	Edema	Eryth.	Edema	Eryth.	Edema	Eryth.	Edema	Eryth.	Edema	Eryth.	Edema
9122	Male	1	1	2	1	2	1	1	1	0	0	0	0
9124		2	1	2	1	2	2	1	2	1	0	0	0
9126		1	0	1	1	1	1	1	0	0	0	0	0
9130		0	0	0	0	1	0	0	0	0	0	0	0
9134		1	0	0	0	1	0	1	0	0	0	0	0
9121	Female	0	0	0	0	0	0	0	0	0	0	0	0
9125		0	0	0	0	0	0	0	0	0	0	0	0
9127		1	0	0	0	0	0	0	0	0	0	0	0
9129		2	2	2	1	2	2	2	2	1	0	0	0
9131		2	2	1	1	1	1	1	0	0	0	0	0

Scores erythema (Eryth.)/edema : 0 no erythema/edema, 1: very slight erythema/edema (barely perceptible), 2: well defined, 3: moderate, 4: severe

The body weight gains were within the range expected for rats used in this type of study and are therefore considered not indicative of toxicity. No macroscopic abnormalities were found in the animals upon necropsy.

Conclusion: The acute percutaneous LD_{50} of CGA-237218 technical FL 891267 to rats was greater than 2020 mg/kg bw. The preparation does not classify as being toxic or harmful on the basis of its acute percutaneous toxicity.

Eye irritation

Report : IIM 5.5.1/05 [redacted] (1991b) CGA-237218 technical FL 891267: Primary eye irritation in rabbits with a microbial pest control agent (MPCA),

Unpublished Report-no. 7013-90

Guideline: EPA 152A-14

GLP: Yes

Materials and Methods: The study was conducted between 23.04. – 30.04.1990 at [redacted]

In a primary eye irritation study 100 mg of CGA-237218 technical FL 891267 (2.9×10^7 CFU per animal) was instilled into the conjunctival sac of one eye of each of 6 young adult albino rabbits (three male and three female). Eye irritation was scored using the Draize scheme for eyes.

Findings: The test substance did not cause any acute systemic toxicological signs or mortality. Individual scorings for ocular reactions after instillation local effects of CGA-237218 technical FL 891267 are presented in Table B.6.2.1-2.

Table B.6.2.1-2. Ocular reactions after instillation of CGA-237218 technical FL 891267

Rabbit		**1-male**						**2-male**						**3-male**					
Time		1 h	1 d	2 d	3 d	4 d	7 d	1 h	1 d	2 d	3 d	4 d	7 d	1 h	1 d	2 d	3 d	4 d	7 d
Cornea		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Conjunctiva	Redness	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0
	Chemosis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Discharge	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 5.5.1-2: Ocular reactions after instillation of CGA-237218 technical FL 900814

Rabbit		**4-female**						**5-female**						**6-female**					
Time		1 h	1 d	2 d	3 d	4 d	7 d	1 h	1 d	2 d	3 d	4 d	7 d	1 h	1 d	2 d	3 d	4 d	7 d
Cornea		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Conjunctiva	Redness	2	1	1	1	0	0	1	1	1	1	1	0	1	1	1	1	1	0
	Chemosis	1	1	1	1	0	0	1	1	1	1	1	0	1	1	1	1	1	0
	Discharge	1	1	1	0	0	0	1	1	1	0	0	0	1	1	2	1	0	0

Conclusion: Instillation of 100 mg CGA-237218 technical FL 900814 resulted in slight conjunctival irritation. The test item is not irritating to the eye.

Note RMS: In table 5.51-2: the batch is not FL891267 and the data are not from the Horbert 1991b study. See below the corrected values for the **Ocular reactions after instillation of CGA-237218 technical FL 891267 in females.**

Table B.6.2.1-3 Ocular reactions after instillation of CGA-237218 technical FL 891267 in females

Rabbit		**4-female**						**5-female**						**6-female**					
Time		1 h	1 d	2 d	3 d	4 d	7 d	1 h	1 d	2 d	3 d	4 d	7 d	1 h	1 d	2 d	3 d	4 d	7 d
Cornea		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Conjunctiva	Redness	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Chemosis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Discharge	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

New data 2016

Data provided for first approval are considered acceptable to cover current requirements no new studies are submitted for renewal of Bta GC-91 according to Regulation (EC) No 1107/2009.

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

B.6.2.2 *In vivo* studies in somatic cells

Information from DAR (2012) *Bacillus thuringiensis* (serotype 1: a constant exotoxin producer; serotype 3: not exotoxin producer) was tested in female rats, fed for three months in drinking water. The animals were sacrificed, the blood samples, the bone marrow cells were analysed. Endpoints analysed: chromosome aberrations in rat bone marrow cells, and in rat peripheral lymphocytes.

Material tested:

autoclaved supernatant of *B. thuringiensis* serotype 1 (AS1);

purified exotoxin received from Shell, UK;

autoclaved supernatant of *Bacillus thuringiensis* serotype 3 (AS3) not producing exotoxin

Results

In the in vivo experiments, rats were exposed to lethal doses of the test materials. Due to the small number of animals (4, 2, 1, respectively) no conclusions can be drawn from the submitted study in bone marrow cells and rat peripheral blood lymphocytes.

The study is inadequate for its evaluation.

New data 2016

No indications of genotoxicity are known for Bta and Btk (please refer to B.6.1.2.3 above). Therefore, studies on genotoxic effects in somatic cells are not considered necessary.

Note RMS: applicant please indicate the reference of the study and present more information in the summary.

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

Information from DAR (2012) Not necessary

New data 2016

No indications of genotoxicity are known for Bta and Btk (please refer to B.6.1.2.3 above). Therefore, studies in genotoxic effects in germ cells are not considered necessary.

B.6.2.4 Summary and conclusions of Tier II studies

See B.6.3 for the overall summary and conclusion.

B.6.3 Summary of mammalian toxicity, pathogenicity and effectiveness and overall evaluation of the active micro-organism

Information from DAR (2012) Laboratory studies on mammalian toxicity of *Bacillus thuringiensis* indicate very little safety risk from direct exposure. No adverse effect and rapid clearance of spores was observed upon oral or pulmonary dosing. Mortality was observed only at high dose levels (> 10^7 CFU per animal) upon systemic administration but no infectivity was observed.

Although *Bacillus thuringiensis* is ubiquitously present in the environment and has been used for decades in plant protection products, only few human health problems have been reported and cases of *Bacillus thuringiensis kurstaki* involved in human clinical infections are extremely rare.

Bacillus thuringiensis has been detected at low levels in nasal swaps and in faeces of workers and residents exposed to *Bacillus thuringiensis*. While an increase in humoral antibodies towards *Bacillus thuringiensis* was detected upon occupational exposure no adverse health effects were noted. No increase in asthma symptoms following aerial exposure to *Bacillus thuringiensis* was recorded in children with asthma.

Bacillus thuringiensis is capable of producing diarrhoeal enterotoxins, but at a level one order of magnitude lower as compared to *Bacillus cereus*. Indeed, no valid evidence has been published linking *Bacillus thuringiensis* with episodes of diarrhoea.

Here, reports on basic laboratory studies with *Bacillus thuringiensis aizawai* are summarised:

Acute toxicity

Acute oral application

Administration of an acute high dose of *Bacillus thuringiensis aizawai* by the oral route induced no adverse effects in rats and mice. *Bacillus thuringiensis aizawai* passes readily through the gastrointestinal tract and was detected only in the faeces with counts rapidly declining. *Bacillus thuringiensis aizawai* remained confined to the gastrointestinal tract and was not systemically distributed and, thus, not detected in the organs.

Upon oral administration of *Bacillus thuringiensis aizawai,* no toxicity related to treatment or pathogenicity was observed and there was no infectivity.

Study type	Test item	Dose level	Findings	NOAEL	Report
Acute oral rat	Bta, CGA-237218 technical, FL 910331	5050 mg per kg b.w. 1.1×10^{10} CFU per kg b.w.	One of ten animals died	$LD_{50} > 5050$ mg per kg b.w.	IIM 5.3.2/01: [redacted] (1991)
Acute oral rat	Bta, CGA-237218 technical	9.4×10^8 CFU per kg b.w.	No adverse effect, no infectivity	$LD_{50} > 9.4 \times 10^8$ per kg b.w.	IIM 5.3.2/02: [redacted] (1990a)

Acute inhalative application

Following inhalative exposure to rats no mortalities were noted in rats at high exposure levels. Upon intratracheal instillation in rats 2 of 36 treated animals died at a dose level of 3.76×10^8 CFU per animal. These effects are related to the mechanical obstructive action of the test material rather then from a pathogenic or infectious activity.

Study type	Test item	Dose level	Findings	NOAEL	Report
Acute intratracheal Rat	Bta, CGA-237218 technical	3.76×10^8 CFU/kg b.w.	2 of 36 animals died, transient signs of toxicity	$LD_{50} > 3.76 \times 10^8$ per kg b.w.	IIM 5.3.3/01: [redacted] (1990b)
Acute inhalation Rat	CGA-237218 WP FL-910986	0.526 and 3.16 mg/L, 5.6 and 37.7 $\times 10^6$ CFU /L	No mortalities, transient clin. signs	$LC_{50} > 3.16$ mg/L 37.7×10^6 CFU /L	IIM 5.3.3/02: [redacted] (1992)

Acute systemic application

Upon intraperitoneal administration in mice no signs of toxicity or infectivity and no mortalities occurred at a dose level of 10^6 per animal. Mortalities at frequencies of 10% and 82% occurred at dose levels of 10^7 and 10^8, respectively.

Upon intraperitoneal administration of *Bacillus thuringiensis aizawai* to mice at dose levels of 10^6 per animal no mortalities or signs of toxicity were noted. A low level of mortalities (10%) occurred at a dose level of 10^7 CFU per animal, while a high frequency of deaths (82%) were observed at the highest dose level. All mortalities occurred by two days post-treatment.

No clinical signs of toxicity and no mortalities were noted in a study upon intravenous administration of 7.6×10^7 CFU CGA-237218 per rat. Infectivity of *Bacillus thuringiensis aizawai*, i.e. invasion and multiplication of micro-organisms could not be demonstrated. Clearance from internal organs was rapid. Only the spleen had significant numbers of the microbe by day 14.

Study type	Test item	Dose level	Findings	NOAEL	Report
Acute intraperitoneal Mouse	Bta, CGA-237218 technical, 91-7288	1.16×10^6 CFU/ mouse	No mortalities	1.16×10^6 CFU per mouse	IIM 5.3.4/01: [redacted] (1992a)
Acute intraperitoneal Mouse	Bta, CGA-237218 technical 911445	2.55×10^6 CFU/ mouse	No toxicity, no infectivity	2.55×10^6 CFU per mouse	IIM 5.3.4/02: [redacted] (1992b)
Acute intraperitoneal Mouse	Bta, CGA-237218 FL-901966 FL-910039 FL-910040 FL-910041 FL-910042	10^8, 10^7, 10^6 CFU/ animal	10^8 CFU/mouse: 82% mortality; 10^7 CFU/mouse: 10% mortality; 10^6 CFU/mouse: no mortality, no toxicity	$LD_{50} > 10^7$ CFU per mouse	IIM 5.3.4/03: [redacted] (1991)
Acute intravenously Rat	Bta, CGA-237218 Technical	7.6×10^7 CFU per rat	No infectivity, no toxicity	7.6×10^7 CFU per rat	IIM 5.3.4/04 [redacted] (1992)

Other acute toxicity endpoints

No mortalities or signs of systemic toxicity were observed upon dermal application of 2020 mg *Bacillus thuringiensis aizawai* /kg b.w. to New Zealand White rabbits for 24 h.

Upon intradermal injection of three different batches of CGA-237218 technical in mice no mortalities or systemic effects were observed. Local effects were observed from extremely irritating to non-irritating.

In a primary eye irritation study, instillation of 0.1 mg CGA-237218 technical FL 891267 (2.9×10^7 CFU *Bacillus thuringiensis aizawai* per animal) in the rabbit eye caused no conjunctival irritation or other ocular effects.

Study type	Test item	Dose level	Findings	NOAEL	Report
Dermal toxicity rat	CGA-237218 technical FL 891267	2020 mg /kg b.w. for 24 h	No systemic effects, slight to well defined edema and erythema	$LD_{50} > 2020$ mg /kg b.w.	IIM 5.5.1/01 [redacted] (1991a)
Subcutaneous mouse	CGA-237218 technical FL 900815	3.8×10^6 CFU/animal	No mortalities extremely irritating	$LD_{50} > 3.8 \times 10^6$ CFU/animal	IIM 5.5.1/02: [redacted] (1991b)
Subcutaneous mouse	CGA-237218 technical FL 900816	2.66×10^6 CFU/animal	No mortalities, slightly irritating	$LD_{50} > 2.66 \times 10^6$ CFU/animal	IIM 5.5.1/03: [redacted] (1991c)
Subcutaneous mouse	CGA-237218 technical FL 900814	1.08×10^6 CFU/animal	No mortalities, non irritating	$LD_{50} > 1.08 \times 10^6$ CFU/animal	IIM 5.5.1/04: [redacted] (1991d)
Eye irritation rabbit	CGA-237218 technical FL 891267	0.1 g (2.9×10^7 CFU) per animal	Non irritating	100 mg	IIM 5.5.1/05 [redacted] (1991b)

Genotoxicity

Suspensions of *Bacillus thuringiensis aizawai* were tested for mutagenic activity in the Ames Salmonella assay. No mutagenic activity was detected in several tester strains with or without metabolic activation by rat liver microsomal fractions.

While cytotoxic concentrations of supernatants from the exotoxin-producing strain (*Bacillus thuringiensis* serotype 1) caused significantly increased chromosomal aberrations in human lymphocytes, no significant clastogenic effect was observed with supernatants from *Bacillus thuringiensis* serotype 3. Similarly, no clastogenic effect

was observed with SA-11 both in vitro in Chinese Hamster cells (CHO) or in vivo in polychromatic erythrocytes in the bone marrow of mice treated with 5000 mg SAN 415 technical (SA-11).

Study type	Assay	Test item	Dose level	Findings	Report
Genotoxicity, In vitro *Salm. typh.*	Microbial gene mutation	CGA 237218 technical 10% in DMSO	19.5 – 5000 µg/plate	Non genotoxic	IIM 5.3.5/01 Hertner (1992)
Genotoxicity, In vitro *Salm. typh.*	Microbial gene mutation	Bt H1, Bt H14 supernatants	0.5 –50 µL, 10-fold concentrated supernatant	Non genotoxic	IIM 5.3.5/02 Carlberg et al. (1995)
Clastogenicity In vitro Human lymphocytes	Chromosomal aberration	*B.thuringiensis*, Serotype 1 or Serotype 3	20% (v/v) of supernatant	Bt 1: Clastogenic at cytotoxic conc., Bt 3: Not clastogenic	IIM 5.5.2/03 Meretoja et al. (1977)

Short term or chronic application

Following thirteen weeks administration of CGA-237218 technical by oral gavage to rats no treatment-related effects was seen on clinical signs, bodyweight gain, ophtalmoscopy, clinical pathology or macroscopic pathology. High counts of *Bacillus thuringiensis* were detected in the caecum but complete clearance was apparent at the end of the 4-week recovery period. The study gave no indication of direct toxicity, infectivity or pathogenicity of *Bacillus thuringiensis aizawai* in the rat upon short term repeated oral administration.

Study type	Test item	Dose level	Findings	NOAEL	Report
90 days, oral rat	Bta, CGA-237218 technical	10^8 CFU per animal per day for 13 weeks	No adverse effects	10^8 CFU per animal per day	IIM 5.3.7.1/01 [redacted] (1993)

Overall conclusion

No toxicity or infectivity was noted in experimental studies upon oral, dermal or inhalative exposure even to exceedingly high dose levels. Upon administration of extremely high dose levels by invasive routes (intranasal, intracerebral or intraperitoneal) mortality occurred in laboratory animals. However, lower doses applied by these routes caused no adverse effects.

No specific monitoring data are available for exposure of operators, workers or bystanders to *Bacillus thuringiensis* subsp. *aizawai* strain GC-91.

No models are currently available to estimate exposure to operators, workers or bystanders from microorganisms. However, in the case of *Bacillus thuringiensis* subsp. *aizawai*, no upper limit is required for the acceptable exposure because the product does not raise significant toxicological concerns. Therefore, estimations of exposure are not considered to be necessary.

Taking together the results of these experimental studies, of epidemiological and occupational evidence and the experience from several decades of safe application of *Bacillus thuringiensis aizawai*–based plant protection products it is appropriate to state that there is no concern with regard to human health.

New data 2016

Data provided for the first approval are considered acceptable to cover current requirements. Neither new studies nor substantial new information is submitted for renewal of Bta GC-91 according to Regulation (EC) No 1107/2009.

Taking together the results of experimental studies (**Table B.6.3-1**), of epidemiological and occupational evidence and the experience from several decades of safe application of Bta- and Btk-based plant protection products, it is appropriate to state that there is no concern with regard to human health.

Table B.6.3-1: Overview of experimental studies on oral, dermal, respiratory, intraperitoneal toxicity or genotoxicity after exposure to Bta GC-91

Study type	Species	Test item	Dose level	Findings	NOAEL	Reference
Acute oral	Rat	Bta, CGA-237218 technical, FL 910331	5050 mg per kg b.w. 1.1×10^{10} CFU per kg b.w.	One of ten animals died	LD_{50} > 5050 mg per kg b.w.	OECD: IIM 5.3.2/01
Acute oral	Rat	Bta, CGA-237218 technical	9.4×10^8 CFU per kg b.w.	No adverse effect, no infectivity	LD_{50} > 9.4×10^8 per kg b.w.	OECD: IIM 5.3.2/02
Acute intratracheal	Rat	Bta, CGA-237218 technical	3.76×10^8 CFU/kg b.w.	2 of 36 animals died, transient signs of toxicity	LD_{50} > 3.76×10^8 per kg b.w.	OECD: IIM 5.3.3/01:
Acute inhalation	Rat	CGA-237218 WP FL-910986	0.526 and 3.16 mg/L, 5.6 and 37.7 $\times 10^6$ CFU /L	No mortalities, transient clin. signs	LC_{50} > 3.16 mg/L 37.7 $\times 10^6$ CFU /L	OECD: IIM 5.3.3/02:
Acute intraperitoneal	Mouse	Bta, CGA-237218 technical, 91-7288	1.16×10^6 CFU/ mouse	No mortalities	1.16×10^6 CFU per mouse	OECD: IIM 5.3.4/01
Acute intraperitoneal	Mouse	Bta, CGA-237218 technical 911445	2.55×10^6 CFU/ mouse	No toxicity, no infectivity	2.55×10^6 CFU per mouse	OECD: IIM 5.3.4/02
Acute intraperitoneal	Mouse	Bta, CGA-237218 FL-901966 FL-910039 FL-910040 FL-910041 FL-910042	10^8, 10^7, 10^6 CFU/ animal	10^8 CFU/mouse: 82% mortality; 10^7 CFU/mouse: 10% mortality; 10^6 CFU/mouse: no mortality , no toxicity	LD_{50} > 10^7 CFU per mouse	OECD: IIM 5.3.4/03
Acute intravenously	Rat	Bta, CGA-237218 Technical	7.6×10^7 CFU per rat	No infectivity, no toxicity	7.6×10^7 CFU per rat	OECD: IIM 5.3.4/04
Dermal toxicity	Rat	CGA-237218 technical FL 891267	2020 mg /kg b.w. for 24 h	No systemic effects, slight to well defined edema and erythema	LD_{50} > 2020 mg /kg b.w.	OECD: IIM 5.5.1/01
Subcutaneous	Mouse	CGA-237218 technical FL 900815	3.8×10^6 CFU/animal	No mortalities extremely irritating	LD_{50} > 3.8×10^6 CFU/animal	OECD: IIM 5.5.1/02
Subcutaneous	Mouse	CGA-237218 technical FL 900816	2.66×10^6 CFU/animal	No mortalities, slightly irritating	LD_{50} > 2.66×10^6 CFU/animal	IIM 5.5.1/03
Subcutaneous	Mouse	CGA-237218 technical FL 900814	1.08×10^6 CFU/animal	No mortalities, non irritating	LD_{50} > 1.08×10^6 CFU/animal	OECD: IIM 5.5.1/04

Study type	Species	Test item	Dose level	Findings	NOAEL	Reference
Eye irritation	Rabbit	CGA-237218 technical FL 891267	0.1 g (2.9×10^7 CFU) per animal	Non irritating	100 mg	OECD: IIM 5.5.1/05
90 days, oral	Rat	Bta, CGA-237218 technical	10^8 CFU per animal per day for 13 weeks	No adverse effects	10^8 CFU per animal per day	OECD: IIM 5.3.7.1/01

B.6.4 References relied on

Literature search
A literature search with regard to human toxicology was performed (Seehase, 2016).

This report summarises the search and selection process of open peer-reviewed literature for Literature review on *Bacillus thuringiensis* subsp. *aizawai* strain GC-91 and *Bacillus thuringiensis* subsp. *kurstaki*, as the *Bacillus thuringiensis* subsp. *aizawai* strain under evaluation is a transconjugant between two *Bacillus thuringiensis* strains affiliating with these two subspecies.

For details regarding the search strategy and the results obtained, please refer to Point 4 and Point 5 of the Literature Review Report.

The selection process resulted in the classification of the available reports in the four categories recommended by the EFSA (2011)[3] guidance document:

a) Studies that are relevant to the data requirement and that provide data for establishing or refining risk assessment parameters. These studies should be considered for reliability.
b) Studies that are relevant to the data requirement, but in the opinion of the applicant provide only supplementary information that does not alter existing risk assessment parameters.
c) Studies for which relevance cannot be clearly determined.
d) Studies of no relevance.

The relevance criteria applied are reported in point 3.2 of this Literature Review Report.
The reliability assessment for relevant studies was done according to the recommendations of the EFSA (2011)[1].

The overall results are shown below in **Table B.6.4-1**.

Table B.6.4-1 Results of the study selection process for Toxicology

Data requirement capture in the search (Toxicology):	**n**
Total number of summary records retrieved after all searches of peer-reviewed literature	51
Number of summary records excluded from the search after rapid assessment for relevance	36
Total number of full-text documents assessed in detail	15
Number of studies excluded from further consideration after detailed assessment of relevance	3
Number of studies not excluded for relevance after detailed assessment	12
Number of studies considered to change the list of endpoints	0

Criteria for relevance and reliability

The criteria for relevance and eligibility used are summarised below in **Table B.6.4-2**

[3] 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

Table B.6.4-2 Criteria for relevance and eligibility used in the review according to Regulation (EC) 1107/2009

Relevance criteria
• Identification of the test species as *Bacillus thuringiensis* subsp. *aizawai* or *B. thuringiensis* subsp. *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* subsp. *aizawai* or *kurstaki* used as microbial pest control agent? • Metabolites or toxins of toxicological concern produced by *Bacillus thuringiensis* subsp. *aizawai* or *kurstaki*
Reliability criteria
• Minimum information reported e.g.: test item or related compound, test species relevant • Clear and comprehensive description of material and methods, including duration, replicates, test conditions • Definition of endpoints • Presentation of results • Guideline compliance

Search methods and results

The literature research was conducted 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 (Table B.6.4-3). Search strategy aimed to find all recent (from 2006 onwards) references that are of toxicological relevance.

Table B.6.4-3 Bibliographic databases used in the search

Database	**BA00**	**CV72**	**SCISEARCH**	**MEDLINE**
Description of database Justification for choosing the source	BIOSIS Previews covers worldwide literature in the field of biology (zoology, botany, microbiology), human and veterinary medicine, biochemistry, pharmacology, toxicology, and environmental sciences, especially from Northern America and Europe. It corresponds to the printed Biological Abstracts and Biological Abstracts/ RRM (Reports, Reviews, Meetings). BA00: 2000 to date	CAB Abstracts covers worldwide literature of agriculture and related sciences including biotechnology, veterinary medicine, nutrition, medicine and forestry sciences. Sources include approx. 9000 international journals, books, conference proceedings, and patents. CV72: 1972 to date	SciSearch covers worldwide literature in the fields of science, technology, and medicine. The database contains all citations published in "Science Citation Index Expanded". Sources include approx. 6650 international journals of 150 disciplines including Clinical Medicine and Life Sciences. IS00: 2000 to date	MEDLINE (Medical Literature Analysis and Retrieval System Online) covers worldwide literature on every area of medicine, including dental medicine, veterinary medicine, psychology, and public health. The database corresponds to the printed "Index Medicus" and to some other printed material. Sources include approx. 4800 international journals. ME00: 2000 to date

Keywords used:

Search strategies aimed to find references addressing the potential pathogenicity, infectivity and toxicity of *B. thuringiensis* subsp. *aizawai* or *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 RA for human health for microorganisms used as plant protection products[4] was considered.

Pathogenicity keywords:

"allergic reaction", adhesion, bacteraemia, carcinogenesis, chronic, coloni/sation/se/zation/ze, deadly, disease, fatal, "histopathological change", illness, immunopathology, infection, inflammation, invasion, lethal, myc/osis/etoma, oncogenesis, pathogen/icity/ic/, persistence, sepsis, tumor/igenesis, virulen/ce/t

Infectivity keywords:

Contagious, epidemic, "high-risk group", immun/e/ocompromised, nosocomial, infect/ious/ive/ivity, "infectious dose", opportunistic, transmissible, transmission

Toxicity keywords:

"biologically active compound", allerg/y/ic/enicity, contaminant/ive, "toxic product", poison/ing/ous, nocuous, noxious, (geno/entero)tox/ic/in/icity/genic, sensiti/zation/sation/sing/zing

General keywords:

Bacillus thuringiensis, Bt, aizawai, kurstaki, human, mammal, mammalian, metabolite

Metabolites:

bacillomycin, cereulide, chitinase, cry(stal) protein(s), cytotoxin, enterotoxin, entomocin, fengycin, glucanase, hbl, hemolysin, cytolytic protein(s), iturin, leucocidin, mycosubtilin, nhe, surfactin, thuringiensin, vegetative insecticide, zwittermycin

Products and strains:

GC-91, Turex, Agree

Details on the search strategies and resulting numbers of retrieved records for *Bacillus thuringiensis* subsp. *aizawai* and *B. thuringiensis* subsp. *kurstaki* are presented in Table B.6.4-2 and Table B.6.4-3.

[4] *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*

Table B.6.4-4 Search for published data on *Bacillus thuringiensis* subsp. *aizawai* and *kurstaki*

Date of the search	13.05.2016
Date span of the search	2006 – 2016
Language limit	English, Spanish, German, French
Other limits set	General concepts: human, animal
Search type	Advanced Search, in all text fields

Table B.6.4-5 Search results

Search strategy	Search terms	Number of hits	After removal of duplicates*
1	((((((((((FT=?tox? OR FT=sensiti?) OR FT=allerg?) OR FT=metabolite) OR FT=contaminant?) OR FT=biologically active compound) OR FT=toxic product) OR FT=poison?) OR FT=nocuous) OR FT=noxious))	3549517	
2	(((((((((FT=contagious OR FT=epidemic) OR FT=high risk group) OR FT=immun?) OR FT=nosocomial) OR FT=infect?) OR FT=infectious dose) OR FT=opportunistic) OR FT=transmissible) OR FT=transmission)	4883771	
3	((((((((((((((FT=immunopathology OR FT= infection) OR FT= inflammation) OR FT=invasion) OR FT=lethal) OR FT= myc?) OR FT=oncogenesis) OR FT=pathog?) OR FT=persistence) OR FT= sepsis) OR FT=tumor?) OR FT=virulen?)))))	5170096	
4	((((((((((((FT=allergic reaction OR FT=adhesion) OR FT=bacteremia) OR FT=carcinogenesis) OR FT=chronic) OR FT=coloni?) OR FT=deadly) OR FT=disease) OR FT=fatal) OR FT="histopathological change") OR FT= illness))))	7102177	
5	((((FT=Bacillus thuringiensis OR FT=Bt) AND FT=aizawai#)))	230	
6	((FT=human OR FT=mammalian) OR FT=mammal)	6560593	
7	1 OR 2 OR 3 OR 4	11665873	
8	5 AND 6 AND 7	11	**8**
9	(((((((FT=leucocidin OR FT=mycosubtilin) OR FT=nhe) OR FT=surfactin) OR FT=thuringiensin) OR FT=vegetative insecticida) OR FT=cry?) OR FT=zwittermycin)	395213	
10	(((((((((((((FT=bacillomycin OR FT=cereulide) OR FT=chi?) OR FT=crystal protein#) OR FT=cytotoxin) OR FT=enterotoxin) OR FT=entomocin) OR FT=fengycin) OR FT=glucanase) OR FT=hbl) OR FT=hemoly?) OR FT=cytolytic protein#) OR FT=iturin))	3364565	
11	9 OR 10	3370358	
12	5 AND 6 AND 11	6	**6**
13	(((((((FT=GC-91 OR FT=GC 91) OR FT=GC91) OR FT=Turex) OR FT=Agree)) AND FT=Bacillus thuringiensis))	37	
14	6 AND 13	5	**2**
15	((((FT=Bacillus thuringiensis OR FT=Bt) AND FT=kurstaki#)))	1509	
16	6 AND 7 AND 15	48	**39**
17	6 AND 11 AND 15	18	**18**
Total number of hits			**52****

= maximum truncation; ? = variable truncation

* Duplicate references identified by the title in several databases were deleted from the list.
** Duplicate references identified by the title in several databases and through several searches (strategy 8, 12, 14 and 16, 17) were deleted from the list.

Results of the study selection process

The relevance criteria were applied to sort out references that could be relevant (*rapid assessment* based on titles and abstracts). In addition, patents and references regarding published abstracts of poster presentations or oral contributions in conferences that were not subjected to peer review were not further considered.
Results were selected by reading and sorting all entries.
After the *rapid assessment* 15 references were identified as being potentially relevant and subjected to a *detailed assessment* of full-text documents.

Below are presented the relevant records identified as relevant upon full text evaluation (Table B.6.4-06 and Table B.6.4-07) and records identified as not relevant upon full text evaluation (Table B.6.4-08).

RMS comments: The literature study is conducted according to the EFSA guidance and acceptable.

Table B.6.4-6 List of bibliographical references of documents identified as relevant upon full text evaluation (sorted by data point)

Data point	Authors	Year	Bibliographic data	Title	Relevant to the data requirement? y/n	Impact on List of Endpoint y/n	Reliability y/n/n.a.
MA 2.8	Berlitz, D.L., Giovenardi, M., Fiuza, L.M.	2006	Neotropical Biology and Conservation, 1(1), pp. 35-38	Toxicology effects of delta-endotoxins and beta-exotoxins of *Bacillus thuringiensis* in Wistar rats	y (supporting information)	n	y
MA 2.8	Kim, M.J., Han, J.K., Park, J.S., Lee, J.S., Lee, S.H., Cho, J.I., Kim, K.S.	2015	J. Microbiol. Biotechnol, 25(6), 872–879	Various enterotoxin and other virulence factor genes widespread among *Bacillus cereus* and *Bacillus thuringiensis* strains	y (supporting information)	n	y
MA 2.8	Obeidat, M., Khyami-Horani, H., Al-Momani, F.	2012	African Journal of Biotechnology 11(46), pp. 10504-10512	Toxicity of *Bacillus thuringiensis* beta-exotoxins and delta-endotoxins to *Drosophila melanogaster*, *Ephestia kuhniella* and human erythrocytes	y (supporting information)	n	y
MA 2.8	Onose, J, Imai, T, Hasumura, M, Ueda, M, Ozeki, Y, Hirose, M.	2008	Food Chem Toxicol., 46(6), pp. 2184-2189	Evaluation of subchronic toxicity of dietary administered Cry1Ab protein from *Bacillus thuringiensis* var. *kurustaki* HD-1 in F344 male rats with chemically induced gastrointestinal impairment	y (supporting information)	n	y
MA 2.8	Shimada, N., Miyamoto, K., Kanda, K., Murata, H.	2006	In Vitro Cellular & Developmental Biology – Animal, 42(1), pp. 45-49	*Bacillus thuringiensis* insecticidal CrylAb toxin does not affect the membrane integrity of the mammalian intestinal epithelial cells: An in vitro study	y (supporting information)	n	y
MA 5.1	Levin, D.B. Eds.: Hajek, A.E., et al.	2009	In: Use of Microbes for Control and Eradication of Invasive Arthropods, Chapter 16, pp. 291-303	Human health effects resulting from exposure to *Bacillus thuringiensis* applied during insect control programmes	y (supporting information)	n	n.a.

Data point	Authors	Year	Bibliographic data	Title	Relevant to the data requirement? y/n	Impact on List of Endpoint y/n	Reliability y/n/n.a.
MA 5.1.2, MA 5.1.3	Baelum, J., Larsen, P., Doekes, G., Sigsgaard, T.	2012	Ann Agric Environ Med., 19(4), pp. 631-636	Health effects of selected microbiological control agents. A 3-year follow-up study	y	n	y
MA 5.1.3	Hansen, V.M., Eilenberg, J., Madsen, A.M.	2010	Biocontrol Science and Technology, Vol. 20, No. 6, pp. 605-619	Occupational exposure to airborne *Bacillus thuringiensis kurstaki* HD1 and other bacteria in greenhouses and vegetable fields	y	n	y
MA 5.2.2.1	Wilcks, A., Hansen, B.M., Hendriksen, N.B., Licht, T.R.	2006	FEMS Immunol Med Microbiol. 48(3), pp. 410-418	Persistence of *Bacillus thuringiensis* bioinsecticides in the gut of human-flora-associated rats	y	n	y
MA 5.2.2.2	Tayabali, A.F., Nguyen, K.C., Seligy, V.L.	2011	Toxicological & Environmental Chemistry, Vol. 93 (1), pp. 314-331	Early murine immune responses from endotracheal exposures to biotechnology-related Bacillus strains	y	n	y
MA 5.2.2.2, MA 5.2.5.1	Barfod, K.K., Poulsen, S.S., Hammer, M., Larsen, S.T.	2010	BMC Microbiol, 10, pp.233	Sub-chronic lung inflammation after airway exposures to *Bacillus thuringiensis* biopesticides in mice	y	n	y
MA 5.2.3	Grisolia, C.K., Oliveira-Filho, E.C., Ramos, F.R., Lopes, M.C., Muniz, D.H., Monnerat, R.G.	2009	Ecotoxicology, 18(1), pp. 22-26	Acute toxicity and cytotoxicity of *Bacillus thuringiensis* and *Bacillus sphaericus* strains on fish and mouse bone marrow	y	n	y

Table B.6.4-7 List of bibliographical references of documents identified as relevant upon full text evaluation (sorted by author)

Authors	Data point	Year	Bibliographic data	Title	Relevant to the data requirement? y/n	Impact on List of End-point y/n	Reliability y/n/n.a.
Baelum, J., Larsen, P., Doekes, G., Sigsgaard, T.	**MA 5.1.2, MA 5.1.3**	2012	Ann Agric Environ Med., 19(4), pp. 631-636	Health effects of selected microbiological control agents. A 3-year follow-up study	y	n	y
Berlitz, D.L., Giovenardi, M., Fiuza, L.M.	**MA 2.8**	2006	Neotropical Biology and Conservation, 1(1), pp. 35-38	Toxicology effects of delta-endotoxins and beta-exotoxins of *Bacillus thuringiensis* in Wistar rats	y (supporting information)	n	y
Barfod, K.K., Poulsen, S.S., Hammer, M., Larsen, S.T.	**MA 5.2.2.2, MA 5.2.5.1**	2010	BMC Microbiol, 10, pp.233	Sub-chronic lung inflammation after airway exposures to *Bacillus thuringiensis* biopesticides in mice	y	n	y
Grisolia, C.K., Oliveira-Filho, E.C., Ramos, F.R., Lopes, M.C., Muniz, D.H., Monnerat, R.G.	**MA 5.2.3**	2009	Ecotoxicology, 18(1), pp. 22-26	Acute toxicity and cytotoxicity of *Bacillus thuringiensis* and *Bacillus sphaericus* strains on fish and mouse bone marrow	y	n	y
Hansen, V.M., Eilenberg, J., Madsen, A.M.	**MA 5.1.3**	2010	Biocontrol Science and Technology, Vol. 20, No. 6, pp. 605-619	Occupational exposure to airborne *Bacillus thuringiensis kurstaki* HD1 and other bacteria in greenhouses and vegetable fields	y	n	y
Kim, M.J., Han, J.K., Park, J.S., Lee, J.S., Lee, S.H., Cho, J.I., Kim, K.S.	**MA 2.8**	2015	J. Microbiol. Biotechnol, 25(6), 872–879	Various enterotoxin and other virulence factor genes widespread among Bacillus cereus and Bacillus thuringiensis strains	y (supporting information)	n	y
Levin, D.B. Eds.: Hajek, A.E., et al.	**MA 5.1**	2009	In: Use of Microbes for Control and Eradication of Invasive Arthropods, Chapter 16, pp. 291-303	Human health effects resulting from exposure to *Bacillus thuringiensis* applied during insect control programmes	y (supporting information)	n	n.a.
Obeidat, M., Khyami-Horani, H., Al-Momani, F.	**MA 2.8**	2012	African Journal of Biotechnology 11(46), pp. 10504-10512	Toxicity of *Bacillus thuringiensis* beta-exotoxins and delta-endotoxins to *Drosophila melanogaster*, *Ephestia kuhniella* and human erythrocytes	y (supporting information)	n	y

Authors	Data point	Year	Bibliographic data	Title	Relevant to the data requirement? y/n	Impact on List of End-point y/n	Reliability y/n/n.a.
Onose, J, Imai, T, Hasumura, M, Ueda, M, Ozeki, Y, Hirose, M.	**MA 2.8**	2008	Food Chem Toxicol., 46(6), pp. 2184-2189	Evaluation of subchronic toxicity of dietary administered Cry1Ab protein from *Bacillus thuringiensis* var. *kurustaki* HD-1 in F344 male rats with chemically induced gastrointestinal impairment	y (supporting information)	n	y
Shimada, N., Miyamoto, K., Kanda, K., Murata, H.	**MA 2.8**	2006	In Vitro Cellular & Developmental Biology – Animal, 42(1), pp. 45-49	*Bacillus thuringiensis* insecticidal CrylAb toxin does not affect the membrane integrity of the mammalian intestinal epithelial cells: An in vitro study	y (supporting information)	n	y
Tayabali, A.F., Nguyen, K.C., Seligy, V.L.	**MA 5.2.2.2**	2011	Toxicological & Environmental Chemistry, Vol. 93(1), pp. 314-331	Early murine immune responses from endotracheal exposures to biotechnology-related Bacillus strains	y	n	y
Wilcks, A., Hansen, B.M., Hendriksen, N.B., Licht, T.R.	**MA 5.2.2.1**	2006	FEMS Immunol Med Microbiol. 48(3), pp. 410-418	Persistence of *Bacillus thuringiensis* bioinsecticides in the gut of human-flora-associated rats	y	n	y

Table B.6.4-8 List of bibliographical references of documents identified as not relevant upon full text analysis (alphabetically by first author)

Authors	Year	Bibliographic data	Title	Abstract	Justification
Lemos, A.J., Siqueira, H.A., Wanderley-Teixeira, V., Maia, F.C., Teixeira, Á.A., Silva, E.J., Oliveira, J.V	2013	Exp Toxicol Pathol, 65(5), pp. 489-95	Effect of sub-lethal doses of *Bacillus thuringiensis* subsp. *aizawai* and deltamethrin with regard to fertility and organ toxicity in pregnant albino rats	Products *with Bacillus thuringiensis* (Bt) and synthetic insecticides have been widely used against important vectors of human diseases. However, few studies have addressed the application of these substances on the female reproduction apparatus during pregnancy at doses that do not cause clinical symptoms of intoxication. Seventy pregnant albino rats were analyzed with regard to fertility and histopathology of the kidneys, liver and lungs as well as the morphology of the neonates. The rats were submitted to three sub-lethal doses of the biological insecticide XenTari® WG (*B. thuringiensis* subsp. *aizawai*) and the synthetic insecticide deltamethrin (Decis® 25CE). After the confirmation of copulation, the insecticides were administered orally for either seven days or during the entire pregnancy. The analysis revealed histopathological alterations in all organs analyzed in both treatments. No miscarriages occurred and the neonates did not exhibit signs of malformation of the head, limbs, thorax or abdomen. However, there were a smaller number of pups in the groups that received higher doses of the insecticides in comparison to the control group. Both insecticides produced similar lesions in the kidneys, liver and lungs and reduced the fertility of rats when administered at sub-lethal doses with no clinical signs of intoxication. Thus, this study suggests that sublethal doses of both insecticides can provide chronic toxicity in humans.	Following oral administration to pregnant rats, the insecticide XenTari® WG (*B. thuringiensis* subsp. *aizawai*) was found to cause kidney, liver and lungs lesions at the highest dose tested. This non-GLP, non-guideline conform study is considered not relevant. Briefly, an unspecified placebo was used as control and not the inactivated bacterium. Moreover, a group size of 10 animals is too small to assess effects on reproductive toxicity. Additionally, histopathological analysis is insufficient as no histopathological peer-review was conducted.
Mezzomo, B.P., Miranda-Vilela, A.L., Grisolia, C.K.	2015	Toxins (Basel)., 7(12), pp. 5348-5358	Toxicological Evaluation of a Potential Immunosensitizer for Use as a Mucosal Adjuvant - *Bacillus thuringiensis* Cry1Ac Spore-Crystals: A Possible Inverse Agonist that Deserves Further Investigation	In addition to their applicability as biopesticides, *Bacillus thuringiensis* (Bt) Cry1Ac spore-crystals are being researched in the immunology field for their potential as adjuvants in mucosal and parenteral immunizations. We aimed to investigate the hematotoxicity and genotoxicity of Bt spore-crystals genetically modified to express Cry1Ac individually, administered orally (p.o.) or with a single intraperitoneal (i.p.) injection 24 h before euthanasia, to simulate the routes of mucosal and parenteral immunizations in Swiss mice. Blood samples were used to perform hemogram, and bone marrow was used for the micronucleus test. Cry1Ac presented cytotoxic effects on erythroid lineage in both routes, being more severe in the i.p. route, which also showed genotoxic effects. The greater severity noted in this route, mainly at 6.75 mg/kg, as well as the	Mice were exposed to a single dose of Cry1Ac administered either *per os* or intraperitoneally. Cry1Ac presented cytotoxic effects on erythroid lineage in both routes, being more severe in the i.p. route, which also showed genotoxic effects. According to OECD test guideline 474, an intraperitoneal injection to assess genotoxic potential of a substance is generally not recommended since it is not an intended route of human exposure. Moreover, the TG 747 recommends two or more treatments administered at 24-hour intervals. Single treatments can be

				intermediate effects at 13.5 mg/kg, and the very low hematotoxicity at 27 mg/kg, suggested a possible inverse agonism. The higher immunogenicity for the p.o. route, particularly at 27 mg/kg, suggested that at this dose, Cry 1Ac could potentially be used as a mucosal adjuvant (but not in parenteral immunizations, due to the genotoxic effects observed). This potential should be investigated further, including making an evaluation of the proposed inverse agonism and carrying out cytokine profiling.	administered, if scientifically justified, e.g. test chemicals known to block cell cycle. This is not the case for cry proteins. Moreover, if animals are treated with the test substance once, samples of bone marrow should be taken at least twice (from independent groups of animals). Due to the lacks in experimental procedure, the presented study is considered not relevant.
Abdel-Al, A.E., Osman, H.H., Ryad, N.F.	2011	Egyptian Journal of Biological Pest Control, 21(2), pp. 203-208	Effect of Teflubenzuron and *Bacillus thuringiensis* on Some Haematological Parameters of Cotton Leaf Worm, *Spodoptera littoralis* (Boisd.) and Albino Rats	A study to evaluate the toxicological and hematological effects of the entomopathogen (*Bacillus thuringiensis kurstaki*) and the insect growth regulator (Teflubenzuron) on the cotton leaf worm, *Spodoptera littoralis* (Boisd.) under laboratory conditions, as well their effects on the population % of the pest in Egyptian clover fields and also to evaluate their toxicity on some hematological parameters of the albino rats was carried out. Obtained results indicated that total haemocyte counts (THC) of *S. littoralis* 6^{th} instar treated in 4^{th} instar with the chitin synthesis inhibitor Teflubenzuron was significantly increased compared to the control. While in Bt., the THC was slightly decreased (6.8%) ($P > 0.05$) compared to the control. Teflubenzuron affected some types of blood cells. It significantly decreased the number of oenocytoids, whereas plasmatocytes were significantly ($P > 0.001$) increased. Meanwhile, Bt. increased insignificantly the number of prohaemocytes and spherulocytes, where plasmatocytes, granulocytes were slightly decreased compared to the control. Application of *Bt. kurstaki* for 12 weeks to rats at dosages of 10000 mg/kg/day did not produce toxic effects. The effect of Bt. showed insignificant changes in body weight, liver, kidney and testicular weights, compared to the levels in the control. On the contrary, Teflubenzuron caused a significant decrease in body and kidney weight of rats and increased liver weight. In addition, there was a slightly decrease in testicular weight compared to the control. It was concluded that Bt. had not any significant effect on the haematological parameters.	Full article not available in libraries or online. Therefore, we contacted the authors on 03.05.16 for a reprint, but received no answer. In addition, according to the available abstracts, there were no effects observed in rats upon administration of *Bt. kurstaki* for 12 weeks at dosages of 10000 mg/kg/day

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KMA 5.1/02	Siegel, J.P.	2001	THE MAMMALIAN SAFETY OF BACILLUS THURINGIENSIS-BASED INSECTICIDES not available, not applicable Journal of invertebrate Pathology, 77, 13-21 GLP/GEP: no Published: yes	no	no	not protected	-	Y KIIM 5.1
	EUROPEAN COMMISSION	2008	, REVIEW REPORT FOR THE ACTIVE SUBSTANCE BACILLUS THURINGIENSIS SPP. AIZAWAI, STRAIN GC-91, SANCO/1538/08 – REV. 4, 13.12.2013				-	
	EFSA	2011	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	no	no	Not protected	-	
KMA 5.1/01 (KMA 5.1/07)	Seehase, S.	2016	LITERATURE REVIEW ON BACILLUS THURINGIENSIS SUBSP. AIZAWAI GC-91: TOXICOLOGY Certis USA LLC, 2281385-MA-05-01 GAB Consulting GmbH, Stade, Germany GLP/GEP: no Published: no	no	yes	New data for active ingredient, not previously submitted nor evaluated	CEU	N
KMA 5.2.2.1/01 (KMA 5.2.2.1/05)	Wilcks, A., Hansen, B.M., Hendriksen, N.B., Licht, T.R.	2006	PERSISTENCE OF BACILLUS THURINGIENSIS BIO-INSECTICIDES IN THE GUT OF HUMAN-FLORA-ASSOCIATED RATS not available, not applicable FEMS Immunol Med Microbiol, 48, pp. 410-418 GLP/GEP: no Published: yes	yes	no	not protected	-	N

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KMA 5.1.3/01 (KMA 5.1.3/05)	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. not available, not applicable Biocontrol Science and Technology, 20(6), 605-619 GLP/GEP: no Published: yes	no	no	not protected	-	N
KMA 5.1/02 (KMA 5.1/08)	Levin, D.B.	2009	HUMAN HEALTH EFFECTS RESULTING FROM EXPOSURE TO BACILLUS THURINGIENSIS APPLIED DURING INSECT CONTROL PROGRAMMES not available, not applicable Use of Microbes for Control and Eradicatin of Invasive Arthropods, 291-303 GLP/GEP: no Published: yes	no	no	not protected	-	N
KMA 5.1.2/03	Jensen, G.B., Larsen, P., Jacobsen, B.L., Madsen, B., Smidt, L., Andrup, L.	2002	BACILLUS THURINGIENSIS IN FECAL SAMPLES FROM GREENHOUSE WORKERS AFTER EXPOSURE TO B. THURINGIENSIS-BASED PESTICIDES not available, not applicable Applied and Environmental Microbiology, 68, 4900-4905 GLP/GEP: no Published: yes	no	yes	protected	-	Y KIIM 5.2
KMA 5.1.2/04	Dively, C.A.	2006	LONG TERM EXPOSURE OF BTA TO EMPOYEES DURING MANUFACTURE Certis USA LLC, not applicable not available GLP/GEP: no Published: no	no	no	not protected	CEU	Y KIIM 5.2
KMA 5.1.2/01 (KMA 5.1.2/05)	Doak, B.	2016	BTZ MEDICAL VERIFICATION Certis USA LLC, not stated [redacted] GLP/GEP: no Published: no	no	yes	New data for active ingredient, not previously submitted nor evaluated	CEU	N

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KMA 5.1.2/02 (KMA 5.1.2/06)	Baelum, J., Larsen, P., Doekes, G., Sigsgaard, T.	2012	HEALTH EFFECTS OF SELECTED MICROBIOLOGICAL CONTROL AGENTS. A 3-YEAR FOLLOW-UP STUDY not available, not applicable Annals of Agricultural and Environmental Medicine, 19(4), 631-636 GLP/GEP: no Published: yes	no	no	not protected	-	N
KMA 5.1.3/01	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 RESPONSE IN FARM WORKERS AFTER EXPOSURE TO BACILLUS THURINGIENSIS PESTICIDES not available, not applicable Environ Health Perspect, 107, 1-15 GLP/GEP: no Published: yes	no	no	not protected	-	Y KIIM 5.2.1
KMA 5.1.3/03	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 not available, not applicable American Journal of Industrial Medicine, 46, 404-407 GLP/GEP: no Published: yes	no	no	not protected	-	Y KIIM 5.2.1
KMA 5.1.4/01	Pearce, M., Habbick, B., Williams, J., Eastman, M., Newman, M.	2002	THE EFFECTS OF AERIAL SPRAYING WITH BACILLUS THURINGIENSIS KURSTAKI ON CHILDREN WITH ASTHMA not available, not applicable Canadian Journal of Public Health, 93, 21-25 GLP/GEP: no Published: yes	no	no	not protected	-	Y KIIM 5.2.3
KMA 5.1.4/02	Petrie, K., Thomas, M., Broadbent, E.	2003	SYMPTOM COMPLAINTS FOLLOWING AERIAL SPRAYING WITH BIOLOGICAL INSECTICIDE FORAY 48B not available, not applicable New Zealand Med J, 116, 1-7 GLP/GEP: no Published: yes	no	no	not protected	-	Y KIIM 5.2.3

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KMA 5.1.4/04	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 not available, not applicable American Journal of Public Health, 80, 848-852 GLP/GEP: no Published: yes	no	no	not protected	-	Y KIIM 5.2.4
KMA 5.1.4/11	Jackson, S.G., Goodbrand, R.B., Ahmed, R., Kasatiya, S.	1995	BACILLUS CEREUS AND BACILLUS THURINGIENSIS ISOLATED IN A GASTROENTERITIS OUTBREAK INVESTIGATION not available, not applicable Lett Appl Microbiol, 21, 103-105 GLP/GEP: no Published: yes	no	no	not protected	-	Y KIIM 5.2.4
KMA 5.1.4/12	Damgaard, P.H., Granum, P.E., Bresciani, J., Torregrossa, M.V., Eilenberg, J., Valentino, L.	1997	CHARACTERIZATION OF BACILLUS THURINGIENSIS ISOLATED FROM INFECTIONS IN BURN WOUNDS not available, not applicable FEMS Immunol Med Microbiol, 18, 47-53 GLP/GEP: no Published: yes	no	no	not protected	-	Y KIIM 5.2.4
KMA 5.1.4/13	Hernandez, E., Ramisse, F., Ducoureau, J., Cruel, T., Cavallo, J.	1998	BACILLUS THURINGIENSIS SUBSP. KONKUKIAN (SEROTYPE H34) SUPERINFECTION: CASE REPORT AND EXPERIMENTAL EVIDENCE OF PATHOGENICITY IN IMMUNOSUPPRESSED MICE not available, not applicable Journal of Clinical Microbiology, 36 (7), 2138-2139 GLP/GEP: no Published: yes	no	no	not protected	-	Y KIIM 5.2.4
KMA 5.2.2.1/01	[redacted]	1991	CGA-237218 TECHNICAL FL910331: ACUTE ORAL TOXICITY STUDY IN RATS WITH A MICROBIAL PEST CONTROL AGENT (MPCA) Certis USA LLC, 8375-91 [redacted] GLP: yes Published: no	yes	yes	protected	CEU	Y KIIM 5.3.2

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KMA 5.2.2.1/02	[redacted]	1990 a	ACUTE ORAL TOXICITY AND INFECTIVITY/PATHOGENICITY STUDY OF CGA-237218 TECHNICAL (BACILLUS THUR. VAR. AIZAWAI) IN RATS Certis USA LLC, 90341D/CBG 517-1/AC [redacted] GLP: yes Published: no	yes	yes	protected	CEU	Y KIIM 5.3.2
KMA 5.2.2.2/01	[redacted]	1990 b	ACUTE PULMONARY TOXICITY AND INFECTIVITY/PATHOGENICITY STUDY OF CGA-237218 TECHNICAL (BACILLUS THURINGIENSIS VAR. AIZAWAI) IN RATS Certis USA LLC, 90323D/CBG 517-2/AC [redacted] GLP: yes Published: no	yes	yes	protected	CEU	Y KIIM 5.3.3
KMA 5.2.2.2/02	[redacted].	1992	CGA-237218 TECHNICAL FL-911722: ACUTE INHALATION TOXICITY STUDY IN RATS WITH A MICROBIAL PEST CONTROL AGENT (MPCA) Certis USA LLC, 8374-91 [redacted] GLP: yes Published: no	yes	yes	protected	CEU	Y KIIM 5.3.3
KMA 5.2.2.2/01 (KMA 5.2.2.2/03)	Tayabali, A.F., Nguyen, K.C., Seligy, V.L.	2010	EARLY MURINE IMMUNE RESPONSES FROM ENDOTRACHEAL EXPOSURES TO BIOTECHNOLOGY-RELATED BACILLUS STRAINS not available, Not applicable Toxicol Environm Chem, 93(1), 314-331 GLP/GEP: no Published: yes	yes	no	not protected	-	N

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KMA 5.2.2.3/01	[redacted]	1992 a	CGA-237218 TECHNICAL 91-7288: ACUTE INTRAPERITONEAL TOXICITIY/PATHOGENICITY SCREEN IN MICE Certis USA LLC, 8515-91 [redacted] GLP/GEP: no Published: no	yes	yes	protected	CEU	Y KIIM 5.3.4
KMA 5.2.2.3/02	[redacted]	1992 b	CGA-237218 TECHNICAL 911445: ACUTE INTRAPERITONEAL TOXICITY/PATHOGENICITY SCREEN IN MICE Certis USA LLC, 8648-91 [redacted] GLP/GEP: no Published: no	yes	yes	protected	CEU	Y KIIM 5.3.4
KMA 5.2.2.3/03	[redacted]	1991	ACUTE INTRAPERITONEAL TOXICITY/PATHOGENICITY SCREENING STUDIES OF TECHNICAL CGA-237218 IN MICE Certis USA LLC, CBG 517-3 [redacted] GLP: yes Published: no	yes	yes	protected	CEU	Y KIIM 5.3.4
KMA 5.2.2.3/04	[redacted]	1990 c	ACUTE INTRAVENOUS TOXICITY AND INFECTIVITY/PATHOGENICITY STUDY OF CGA-237218 TECHNICAL (BACILLUS THUR. VAR. KURSTAKI) Certis USA LLC, 90324D/CBG 517-3/AC [redacted] GLP: yes Published: no	yes	yes	protected	CEU	Y KIIM 5.3.4
KMA 5.2.3/01	Hertner, T.	1992	SALMONELLA AND ESCHERICHIA/LIVER-MICROSOME TEST Certis USA LLC, 922118 Genetic Toxicology, CIBA-Geigy Ltd., Basle, Switzerland GLP: yes Published: no	no	yes	protected	CEU	Y KIIM 5.3.5

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KMA 5.2.3/02	Meretoja, T., Carlberg, G., Gripenberg, U., Linnainmaa, K., Sorsa, M.	1977	MUTAGENICITY OF BACILLUS THURINGIENSIS EXOTOXIN not available, not applicable Hereditas, journal in the field of genetics and cytogenetics, 85, 105-112 GLP/GEP: no Published: yes	no	no	not protected	-	Y KIIM 5.3.5
KMA 5.2.3/03	Carlberg, G., Tikkanen, L., Abdel-Hameed, A.A.	1995	SAFETY TESTING OF BACILLUS THURINGIENSIS PREPARATIONS, INCLUDING THURINGIENSIN, USING SALMONELLA ASSAY not available, not applicable Journal of invertebrate Pathology, 66, 68-71 GLP/GEP: no Published: yes	no	no	not protected	-	Y KIIM 5.3.5
KMA 5.2.3/01 (KMA 5.2.3/05)	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. not available, not applicable Ecotoxicology, 18(1), 22-26 GLP/GEP: no Published: yes	yes	no	not protected	-	N
KMA 5.2.5/01	[redacted]	1993	CGA-237218 TECHNICAL: THIRTEEN-WEEK ORAL TOXICITY/INFECTIVITY IN RATS Certis USA LLC, CBG 595/930636 [redacted] GLP: yes Published: no	yes	yes	protected	CEU	Y KIIM 5.3.7.1
KMA 5.2.5/02	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 not available, not applicable Fundam Appl Toxicol, 8, 236-242 GLP/GEP: no Published: yes	no	no	not protected	-	Y KIIM 5.3.7.1

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KMA 5.2.5.1/01	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 not available, not applicable BMC Microbiology, 10, 233 GLP/GEP: no Published: yes **Submitted in: KMA 5.2.2.2/02**	yes	no	not protected	-	N
KMA 5.3/01	[redacted]	1991 a	CGA-237218 TECHNICAL FL 891267: ACUTE DERMAL TOXICITY STUDY IN RABBITS WITH A MICROBIAL PEST CONTROL AGENT MCPA) Certis USA LLC, 7012-90 [redacted] GLP: yes Published: no	yes	no	not protected	CEU	Y KIIM 5.5.1
KMA 5.3/02	[redacted]	1990 a	CGA-237218 TECHNICAL FL 900815: MOUSE SUBCUTANEOUS INJECTION Certis USA LLC, 7008-90 [redacted] GLP/GEP: no Published: no	yes	no	not protected	CEU	Y KIIM 5.5.1
KMA 5.3/03	[redacted]	1990 b	CGA-237218 TECHNICAL FL 900816: MOUSE SUBCUTANEOUS INJECTION Certis USA LLC, 7009-90 [redacted] GLP/GEP: no Published: no	yes	no	not protected	CEU	Y KIIM 5.5.1
KMA 5.3/04	[redacted]	1990 c	CGA-237218 TECHNICAL FL 900814: MOUSE SUBCUTANEOUS INJECTION Certis USA LLC, 7007-90 [redacted] GLP/GEP: no Published: no	yes	no	not protected	CEU	Y KIIM 5.5.1

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KMA 5.3/05	[redacted]	1991 b	CGA-237218 TECHNICAL FL 891267: PRIMARY EYE IRRITATION STUDY IN RABBITS WITH A MICROBIAL PEST CONTROL AGENT (MCPA) Certis USA LLC, 7013-90 [redacted] GLP: yes Published: no	yes	no	not protected	CEU	Y KIIM 5.5.1
KMA 5.3/07	Hernandez, E., Ramisse, F., Cruel, T., Vagueresse, R., Cavallo, J.	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 not available, not applicable FEMS Immunol Med Microbiol, 24, 43-47 GLP/GEP: no Published: yes	no	no	not protected	-	Y KIIM 5.5.1
KMA 5.3/08	Hernandez, E., Ramisse, F., Gros, P., Cavallo, J.	2000	SUPER-INFECTION BY BACILLUS THURINGIENSIS H34 OR 3A3B CAN LEAD TO DEATH IN MICE INFECTED WITH INFLUENZA A VIRUS not available, not applicable FEMS Immunol Med Microbiol, 29, 177-181 GLP/GEP: no Published: yes	no	no	not protected	-	Y KIIM 5.5.1