Renewal
Assessment Report

Bacillus thuringiensis ssp. aizawai strain ABTS-1857

- XenTari® WG -

Volume 3 – B.6 Effects on human health

Rapporteur Member State: The Netherlands

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Version history

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B.6 Effects on human health

B.6.1 Basic acute toxicity studies

B.6.1.1 Acute oral toxicity

The following acute oral toxicity and clearance study, performed using ABG-3614 (XenTari® WG), was assessed in the EU Review was summarised in the DAR.

Report:	IIIM 7.1.1/01[redacted] (1991)
Title:	Acute oral toxicity and clearance study in rats with ABG-6314
Document No:	3774-90-0470-TX-001
Guidelines:	Not stated
GLP:	Yes

Abstract: The acute oral toxicity and clearance of ABG-6314 (2 x10^{10} CFU/g) was evaluated in male and female Sprague Dawley rats. A single dose of 5000 mg/kg bw (10 mL/kg bw) was administered to 10 rats by oral gavage. The animals were placed into metabolism cages immediately after dose administration, for the collection of faecal samples daily until Day 6 and then on Days 8, 10, 12 and 14. The animals were observed for mortality and clinical signs at 1, 2.5 and 4 hours after administration and then daily thereafter. Bodyweights were recorded just prior to dosing and on Days 7, 14, 21 and 26. At the end of the 26-day observation period, animals were sacrificed and subjected to gross necropsy. All animals survived the duration of the study. There were no significant clinical observations. There were no treatment-related effects on body weight. The total number of viable colony forming units (CFU) of the test organisms excreted ranged from 1.9-3.8 x 10^{10} per animal on Day 1. For most animals, the total numbers of CFU excreted decreased at each collection point interval thereafter. Clearance ranged from Day 10-24. Decreased faeces were observed during the study period for the animals for which clearance was not established until Days 22 and 24 and may have contributed to the delay in clearance for these animals. No abnormal findings were noted in any animals at necropsy.

Conclusion: The acute oral LD_{50} in the rat of ABG-6314 was found to be greater than 5000 mg/kg bw under the conditions of this study. XenTari® WG does not require classification for acute oral toxicity according to the CLP Regulation.

The study meets the requirements of Part B of Regulation (EU) No 284/2013 and no further data are required for this endpoint.

B.6.1.2 Acute inhalation toxicity

The following acute inhalation toxicity study, performed using ABG-3614 (XenTari® WG), was assessed in the EU Review and was summarised in the DAR.

Report:	IIIM 7.1.3/01, [redacted] (1991)
Title:	Acute inhalation toxicity study with ABG-6314 in rats
Document No:	7788-91
Guidelines:	US EPA Guidelines 152A-12
GLP:	Yes

Abstract: The acute inhalation toxicity of ABG-6314 (2 $x10^{10}$ spores/g) was evaluated in male and female Sprague Dawley rats. Five rats/sex were exposed to aerosols of ABG-6314 at a measured concentration of 3.05 mg/L (5.8 x 10^7 CFU/L), for four hours. Rats were observed for mortality and clinical signs on the day of exposure and at least daily thereafter. Bodyweights were recorded just prior to exposure and on Days 7 and 14. At study termination, all rats were sacrificed and subjected to gross necropsy. A concentration of 5.0 mg/L with 25% of particles <1 µm was not achieved due to the nature of the test material; therefore the maximum attainable concentration was 3.05 mg/l, with an MMAD of 3.44 µm. Observations could not be performed during the exposure period, due to the cloudiness of the test material. All rats survived the duration of the study. Post-exposure observations included decrease activity, lacrimation, nasal discharge, piloerection, polyuria and salivation. These signs were noted only on the day of dosing. No abnormal findings were observed at necropsy.

Conclusion: Under the conditions of the study, the acute inhalation LC_{50} of ABG-6314 in rats was found to be >3.05 mg/L (5.8 x 10^7 CFU/L), the maximum attainable concentration. XenTari® WG does not require classification for acute inhalation toxicity according to the CLP Regulation.

A more recent acute inhalation toxicity study is also available for the related product XenTari® DF.

Report:	MMP 7.1.2/02, [redacted] (2011)
Title:	XenTari DF Acute Inhalation Toxicity Study in Rats
Document No:	33230
Guidelines:	U.S. EPA Health Effects Test Guidelines, OPPTS 870.1300 OECD Guidelines for the Testing of Chemicals, Test No. 403 JMAFF 12-Nousan-8147
GLP:	Yes

Material: XenTari® DF, Lot # 194-275-PG. Composition: 54.0% *Bacillus thuringiensis* ssp. *aizawai*, strain ABTS-1857 fermentation solids, spores and insecticidal toxins.

Methods: Five male and five female Sprague Dawley rats (bodyweight range: 204-263 g, age not stated) were selected for the study. Test animals were placed into polycarbonate restraint tubes, which fitted into the exposure chamber allowing the animals to be exposed via the nose only. The gravimetric and nominal chamber concentrations of the test material were 5.10 mg/L and 20.94 mg/L, respectively. The test animals were exposed for 4 hours. All

animals were observed for mortality during the exposure period and were examined for signs of gross toxicity, and behavioural changes upon removal from the exposure tube and at least once daily thereafter for 14 days. Observations included gross evaluation of skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behaviour pattern. Particular attention was directed to observation of tremors, convulsions, salivation, diarrhoea, and coma

Findings: All animals survived exposure to the test atmosphere. Following exposure, three males and all females exhibited irregular respiration and one female exhibited red ocular discharge, but recovered by Day 4 and appeared active and healthy for the remainder of the 14-day observation period. Although all rats lost body weight by Day 1 and/or 3, all animals showed a continued weight gain thereafter. No gross abnormalities were noted at necropsy.

Conclusion: Under the conditions of this study, the single exposure acute inhalation LC_{50} of XenTari® DF in the rat was >5.10 mg/L. XenTari® WG does not require classification for acute inhalation toxicity according to the CLP Regulation.

The studies meet the requirements of Part B of Regulation (EU) No 284/2013 and no further data are required for this endpoint.

B.6.1.3 Acute percutaneous toxicity

The following acute dermal toxicity study, performed using ABG-3614 (XenTari® WG), was assessed in the EU Review but was not summarised in detail in the DAR.

Report:	IIIM 7.1.2/01[redacted] (1991a)
Title:	Acute dermal toxicity study in albino rabbits with ABG-6314
Document No:	90-0471
Guidelines:	US EPA Guidelines 142A-11
GLP:	Yes

Material: ABG-6314, Lot No. 46-095-BR; potency: 2 $x10^{10}$ spores/g.

Method: Twenty-four hours prior to dosing five male and five female New Zealand White rabbits (bodyweight range 2300-2750 g for males, 2200-2450 g for females) were clipped free of hair on their back and sides. At dosing 2000 mg/kg bw of ABG-6314 was applied undiluted to each rabbit. The dose was administered to an area of approximately 10.2 x 20.3 cm over the back and sides of the rabbit. Prior to administration, the test material was moistened with deionised water to enable it to be applied uniformly. The application site was covered with semi-occlusive dressings and a restraining collar was placed around the neck of each animal. After a 24-hour exposure period, the dressings were removed and the test site skin was rinsed with water to remove residual test material. The animals were observed for mortalities and clinical signs at 1, 2.5 and 4 hours post dosing and then daily for the duration of the observation period. All test sites were scored for erythema, eschar and oedema according to the Draize system. Scores were recorded immediately after the 24 hour exposure period and again on Days 3, 7, 10 and 14. Bodyweights were recorded prior to dosing and again on

Days 7 and 14 of the study. At study termination all animals were sacrificed and subjected to necropsy examinations.

Findings: There were no mortalities. Soft faeces were observed in the rabbits at several intervals during the study. Soft faeces noted mainly on the day of dosing was related to the dosing procedures (bandaging and restraining collar) used at administration. Sporadic incidences of soft faeces were observed in one male rabbit on Day 8, and in one female rabbit on Days 10-12. A creamy white nasal discharge was observed in one rabbit on Days 2 and 3 of the study. These findings were not considered to be treatment related. All rabbits displayed well-defined erythema on Day 1; erythema persisted in all rabbits until Day 7, with one female displaying erythema until Day 10. Slight oedema was noted for one female rabbit only on Day 1 post dosing. All animals gained weight as expected throughout the study. Necropsy did not reveal any abnormalities.

Conclusion: Under the conditions of the study, the acute dermal LD_{50} in the rabbit of ABG-6314 was >2000 mg/kg bw. XenTari® WG does not require classification for acute dermal toxicity according to the CLP Regulation.

The study meets the requirements of Part B of Regulation (EU) No 284/2013 and no further data are required for this endpoint.

B.6.2 Additional acute toxicity studies

B.6.2.1 Skin irritation

The following skin irritation study, performed using ABG-3614 (XenTari® WG), was assessed in the EU Review and was summarised in the DAR.

Report:	IIIM 7.1.4/01, [redacted] 1991b
Title:	Primary dermal irritation study in albino rabbits with ABG-6314
Document No:	90-0473
Guidelines:	US EPA Guideline 152A-11
GLP:	Yes

Abstract: 0.5 g ABG-6314 (moistened with 0.5 mL water) was applied for 4 hours under semi-occlusive conditions to the shorn dorsal skin of six New Zealand White rabbits. Residual test material was removed by washing the application site with water. Local dermal reactions were scored according to the Draize scale at 0.5-1, 24, 48 and 72 hours after patch removal, and at Days 4 and 8. Signs of irritation were limited to Grade 1-2 erythema in all rabbits from the 30-60 minute observation; findings resolved by Day 8.

Conclusion: ABG-6314 was found to be a slight skin irritant under the conditions of this study; no classification for skin irritation is required according to the CLP Regulation.

The study meets the requirements of Part B of Regulation (EU) No 284/2013 and no further data are required for this endpoint.

B.6.2.2 Eye irritation

The following eye irritation study, performed using ABG-3614 (XenTari® WG), was assessed in the EU Review and was summarised in the DAR.

Report:	IIIM 7.1.5/01, [redacted] (1991c)
Title:	Primary eye irritation study in albino rabbits
Document No:	90-0472
Guidelines:	US EPA Guidelines 152A-14
GLP:	Yes

Abstract: 0.9 g ABG-6314 was instilled into one eye of nine New Zealand White rabbits. The treated eyes of six rabbits were unwashed; the treated eyes of three rabbits were washed with water 30 seconds following treatment. Observations for irritation and ocular lesions were performed at 1, 24, 48 and 72 hours post dosing and on Days 4, 7, 10 and 14, according to the Draize scale. In the unwashed group, a 'lacklustre' appearance of the cornea was observed in one rabbit at 24 hour observation; no corneal effects were noted for any of the other animals. Iridial hyperaemia was observed in all rabbits at 1 hour only; this represents a minimal response and is not considered a Draize Grade 1 effect. Conjunctival erythema, chemosis and discharge were observed in all rabbits from 1-72 hours. Minimal erythema persisted in two rabbits to Day 10. Purulent discharge was observed in four rabbits at 24 hours and in five rabbits at 48 hours, persisting in one rabbit to 72 hours. Conjunctival blistering was observed in three rabbits from 24-72 hours. Conjunctival petechial haemorrhage was noted in one rabbit at 48 and 72 hours. No significant conjunctival effects were noted in any of the rabbits after Day 4 of the study. A yellow crusting around the edges of the eyelid was observed in all rabbits at 24 hours, and persisted in one rabbit to 48 hours. In the washed group, no corneal or iridial effects were observed. Conjunctival erythema, chemosis and discharge were observed in all rabbits from 1-72 hours. Minimal erythema persisted in one rabbit to Day 7. No significant conjunctival effects were noted in any of the rabbits after 72 hours. A yellow crusting around the edges of the eyelid was observed in two rabbits at 24 and 48 hours.

Conclusion: The product was found to be an eye irritant under the conditions of this study; classification for eye irritation (Warning H319) is proposed according to the CLP Regulation.

The study meets the requirements of Part B of Regulation (EU) No 284/2013 and no further data are required for this endpoint.

B.6.2.3 Skin sensitisation

The following skin sensitisation study, performed using XenTari (XenTari® WG), was assessed in the EU Review and was summarised in the DAR.

Report:	IIIM 7.1.6/01, [redacted] (1994)
Title:	Maximisation test of XenTari in guinea pigs
Document No:	M94AU50.246
Guidelines:	OECD Guideline 406
GLP:	Yes

Abstract: The skin sensitisation potential of XenTari was investigated in a Maximisation assay. A test group of 20 guinea pigs was treated using intradermal induction application of 2.5% test material and a 48-hour occlusive topical induction application of 25% test material. A control group of 20 guinea pigs was treated using vehicle only. All test and control animals were challenged using a 24-hour occlusive topical application of 5% test material; local dermal reactions were scored at 24, 48 and 72 hours following challenge. No reactions were seen in any of the test animals following the challenge application.

Conclusion: No evidence of skin sensitisation was seen under the conditions of this study. Classification of XenTari® WG is not required according to the CLP Regulation.

The study meets the requirements of Part B of Regulation (EU) No 284/2013 and no further data are required for this endpoint. The available skin sensitisation studies for the active substance and the product both give a negative response; however, as a general principle, it is considered that microbial active substances are potential skin and respiratory sensitisers; therefore all products containing microbial active substances must carry a standard label phrase "contains B. thuringiensis subsp. aizawai. Micro-organisms may have the potential to provoke sensitising reactions."

B.6.3 Data on exposure

Derivation of toxicological reference values (including an AOEL) is not applicable for *Bacillus thuringiensis* ssp. *aizawai* (ABTS 1857) based on the lack of toxicity, infectivity or pathogenicity. Quantitative assessment of operator, worker, bystander and resident exposure is therefore not required for the product XenTari WG.

Some *Bacillus thuringiensis* strains contain the non-haemolytic enterotoxin complex (*nhe*) genes; however the majority of *B. thuringiensis* strains investigated have been shown not to produce enterotoxins. *Bacillus thuringiensis* ssp. *aizawai* (ABTS 1857)) has been shown not to produce β-exotoxin, cytolytic proteins or enterotoxins during the production process. One report (Damgaard, 1995) shows the production of enterotoxin by this strain, but only at relatively low levels and under very specific culture conditions. Culture of this strain in Brain Heart Infusion Broth (BHIB) resulted in an enterotoxin titre of enterotoxin of 23, compared to 1629 in an enterotoxin-producing *B. cereus* strain. It is possible, therefore, that low levels of enterotoxin may be produced by *Bacillus thuringiensis* ssp. *aizawai* (ABTS 1857) under very specific conditions (BHIB). This would be unlikely to occur in practice post-application and, even if the enterotoxin was produced, the low levels represent a significant safety margin (~70x) over the levels of *B. cereus* (10^5 organisms/g food) typically required to cause

food poisoning. The emetic toxin cereulide is not produced by all strains of *B. cereus* and is not produced by *B. thuringiensis*.

It is therefore concluded that the product can be used without potential health risks to operators, workers or bystanders, subject to the use of protective equipment specified on the product label.

B.6.4 Available toxicological data relating to non-active substances

Details of the product co-formulants are included in the confidential information. The non-active substances are toxicologically inert and do not result in any additional classification of the product under the CLP Regulation.

B.6.5 Supplementary studies for combinations of plant protection products

Not relevant: the product is not required to be used in combination with other products.

B.6.6 Summary and evaluation of health effects

Overview of the available data

Study	Test material	Species	Result	Reference
Acute oral toxicity	ABG-6314[a]	Rat	LD_{50} >5000 mg/kg bw	[redacted] (1991)
Acute inhalation toxicity	ABG-6314[a]	Rat	LC_{50} >3.05 mg/L	[redacted] (1991)
Acute inhalation toxicity	XenTari® DF[b]	Rat	LC_{50} >5.10 mg/L	[redacted] (2011)
Acute dermal toxicity	ABG-6314[a]	Rabbit	LD_{50} >2000 mg/kg bw	[redacted] (1991a)
Skin irritation	ABG-6314[a]	Rabbit	Slight irritant	[redacted] (1991b)
Eye irritation	ABG-6314[a]	Rabbit	Irritant	[redacted] (1991c)
Skin sensitisation (Maximisation)	ABG-6314[a]	Guinea pig	Non sensitiser	[redacted] (1994)

[a]*formulation identical to XenTari® WG*
[b]*formulation comparable to XenTari® WG*

The product XenTari® WG (development code ABG-6314) is of low acute oral, dermal and inhalation toxicity. An acute oral LD_{50} of >5000 mg/kg bw is reported in a study in the rat; an acute dermal LD_{50} of >2000 mg/kg bw is reported in a study in the rabbit. No mortality was reported at the maximum attainable concentration of 3.05 mg/L in an acute inhalation toxicity study with XenTari WG. An acute inhalation toxicity study performed with the related product XenTari DF reports an LC_{50} value of 5.10 mg/L. XenTari WG does not therefore require classification for acute toxicity according to the CLP Regulation. XenTari WG is a slight skin irritant, but does not require classification for skin irritation according to the CLP Regulation. Findings in an eye irritation study trigger classification of XenTari WG as an eye irritant (Warning, H319) according to the CLP Regulation. No evidence of skin sensitisation was seen in a Maximisation study. Classification of XenTari WG as a skin sensitiser is not required according to the CLP Regulation; however given the microbial nature of the active substance the product is considered to be a potential sensitiser. The product label must include a standard warning phrase and dermal and inhalation exposure should be minimised through the use of appropriate PPE. The standard warning phrase may need to be reviewed as weight of evidence of years of use of XenTari WG has not presented any sensitization incidents.

Bacillus thuringiensis ssp. *aizawai* (ABTS 1857) is not toxic, infective or pathogenic. The product XenTari WG is of low toxicity but is considered to be a potential skin and respiratory sensitiser. Commercial products manufactured from different strains of *Bacillus thuringiensis* have been applied in agriculture and forestry for crop and tree protection and in waterways for public health over many decades and very few health concerns relating to its use have ever been reported. Despite the widespread use of *Bacillus thuringiensis*-based products, the instances of allergic reaction following exposure are also considered to be very rare.

Derivation of toxicological reference values (including an AOEL) is not applicable for *Bacillus thuringiensis* ssp. *aizawai* (ABTS 1857) based on the lack of toxicity, infectivity or pathogenicity. Quantitative assessment of operator, worker, bystander and resident exposure is therefore not required for the product XenTari WG. It is therefore concluded that the product can be used without potential health risks to operators, workers or bystanders, subject to the use of protective equipment specified on the product label.

Overall, it is concluded that *Bacillus thuringiensis* subsp. *aizawai* Strain ABTS-1857 as a 54% WG formulation can be used in a manner consistent with label recommendations without potential health risks to operators, workers or bystanders

B.6.7 References relied on

See B.6 MA for summary literature search.

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
IIIM 7.1.1/01	[redacted]	1991	Acute oral toxicity and clearance study in rats with ABG-6314 [redacted] Company report No.: 3774-90-0470-TX-001 GLP Unpublished (Previously evaluated in the DAR)	Y	N	-	VBC
IIIM 7.1.3/01	[redacted]	1991	Acute inhalation toxicity study with ABG-6314 in rats [redacted] Company report No.: 7788-91 GLP Unpublished (Previously evaluated in the DAR)	Y	N	-	VBC
MMP 7.1.2 /02	[redacted]	2011	XenTari DF – Acute Inhalation Toxicity Study in Rats [redacted] Company report No.: 33230 GLP Unpublished	Y	Y	Study submitted for the first time	VBC

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protec-tion claimed Y/N	Justification if data protection is claimed	Owner
IIIM 7.1.2/01	[redacted]	1991a	Acute dermal toxicity study in albino rabbits with ABG-6314 [redacted] Company report No.: 90-0471 GLP Unpublished (Previously evaluated in the DAR)	Y	N	-	VBC
IIIM 7.1.4/01	[redacted]	1991b	Primary dermal irritation study in albino rabbits with ABG-6314 [redacted] Company report No.: 90-0473 GLP Unpublished (Previously evaluated in the DAR)	Y	N	-	VBC
IIIM 7.1.5/01	[redacted]	1991c	Primary eye irritation study in albino rabbits with ABG-6314 [redacted]. Company report No.: 90-0472 GLP Unpublished (Previously evaluated in the DAR)	Y	N	-	VBC
IIIM 7.1.6/01	[redacted]	1994	Maximization test of ZenTari in guinea pigs [redacted] Company report No.: M94AU50.246 GLP Unpublished (Previously evaluated in the DAR)	Y	N	-	VBC