Safety evaluation of the food enzyme pullulanase from Pullulanibacillus naganoensis strain AE-PL

Vittorio Silano, Claudia Bolognesi, Laurence Castle, Kevin Chipman, Jean Pierre J. P. Cravedi, Paul Fowler, Roland Franz, Konrad Grob, Rainer Gürtler, Trine Husøy, et al.

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Safety evaluation of the food enzyme pullulanase from *Pullulanibacillus naganoensis* strain AE-PL

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF), Vittorio Silano, Claudia Bolognesi, Laurence Castle, Kevin Chipman, Jean-Pierre Cravedi, Paul Fowler, Roland Franz, Konrad Grob, Rainer Gürtler, Trine Husøy, Sirpa Kärenlampi, Wim Mennes, Maria Rosaria Milana, Karla Pfaff, Gilles Riviere, Jannavi Srinivasan, Maria de Fátima Tavares Poças, Christina Tlustos, Detlef Wölfle, Holger Zorn, Andrew Chesson, Boet Glandorf, Lieve Herman, Klaus-Dieter Jany, Francesca Marcon, André Penninks, Andrew Smith, Davor Želježic, Magdalena Andryszkiewicz, Margarita Aguilera-Gómez, Natalia Kovalkovičová, Yi Liu and Karl-Heinz Engel

Abstract

The food enzyme considered in this opinion is a pullulanase (pullulan 6-α-glucanohydrolase; EC 3.2.1.41) produced with a non-genetically modified *Pullulanibacillus naganoensis* (strain AE-PL) by Amano Enzyme Inc. (Japan). The pullulanase food enzyme is intended to be used in starch processing for the production of glucose syrups. Since residual amounts of total organic solid (TOS) in glucose syrups are removed by filtration and purification during starch processing, dietary exposure assessment was not performed. Genotoxicity tests made with the food enzyme indicated no genotoxic potential. A repeated dose 90-day oral toxicity study in rodents, carried out with the food enzyme, showed minor effects that were considered to be of no biological relevance. The allergenicity was evaluated by comparing the amino acid sequence to those of known allergens and no match was found. The Panel considered that there are no indications for food allergic reactions to dietary intake of this food enzyme. Based on the removal of residual amounts of TOS from glucose syrups, consumer exposure is not expected. In addition, the safety of the manufacturing process, the compositional and biochemical data lead the Panel to conclude that the food enzyme pullulanase from *P. naganoensis* (strain AE-PL) does not give rise to safety concerns under the intended conditions of use.

Keywords: food enzyme, pullulanase, α-dextrin endo-1,6-alpha-glucosidase, EC 3.2.1.41, pullulan α-1,6-glucanohydrolase, *Pullulanibacillus naganoensis*

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1. Introduction

Article 3 of the Regulation (EC) No 1332/2008[1] provides definitions for 'food enzyme' and 'food enzyme preparation'.

'Food enzyme' means a product obtained from plants, animals or microorganisms or products thereof including a product obtained by a fermentation process using microorganisms: (i) containing one or more enzymes capable of catalysing a specific biochemical reaction and (ii) added to food for a technological purpose at any stage of the manufacturing, processing, preparation, treatment, packaging, transport or storage of foods.

'Food enzyme preparation' means a formulation consisting of one or more food enzymes in which substances such as food additives and/or other food ingredients are incorporated to facilitate their storage, sale, standardisation, dilution or dissolution.

Before January 2009, food enzymes other than those used as food additives were not regulated or were regulated as processing aids under the legislation of the Member States. On 20 January 2009, Regulation (EC) No 1332/2008 on food enzymes entered into force. This Regulation applies to enzymes that are added to food to perform a technological function in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food, including enzymes used as processing aids. Regulation (EC) No 1331/2008[2] established European Union (EU) procedures for the safety assessment and the authorisation procedure of food additives, food enzymes and food flavourings. The use of a food enzyme shall be authorised only if it is demonstrated that:

i) it does not pose a safety concern to the health of the consumer at the level of use proposed;
ii) there is a reasonable technological need; and
iii) its use does not mislead the consumer.

All food enzymes currently on the EU market and intended to remain on that market as well as all new food enzymes shall be subjected to a safety evaluation by the European Food Safety Authority (EFSA) and an approval via an EU Community list.

The 'Guidance on submission of a dossier on a food enzyme for evaluation' (EFSA CEF Panel, 2009) lays down the administrative, technical and toxicological data required.

1.1. Background and Terms of Reference as provided by the requestor

1.1.1. Background as provided by the European Commission

Only food enzymes included in the Union list may be placed on the market as such and used in foods, in accordance with the specifications and conditions of use provided for in Article 7 (2) of Regulation (EC) No 1332/2008 on food enzymes.

Five applications have been introduced by the companies 'Amano Enzyme Inc', 'Caglificio Clerici S.p.A.' and 'Danisco US Inc.' for the authorisation of the food enzymes Pullulanase from *Klebsiella pneumoniae* (strain AE-PUL), Pullulanase from *Pullulanibacillus naganoensis* (strain AE-PL), Rhizopuspepsin from *Rhizopus niveus* (strain AE-N), Rennet paste from abomasum of goat (*Capra aegagus hircus*), sheep (*Ovis aries*) and cattle (*Bos primigenius*), Cellulase from a genetically modified strain of *Trichoderma reesei* (DP-Nzc36).

Following the requirements of Article 12.1 of Commission Regulation (EU) No 234/2011 implementing Regulation (EC) No 1331/2008, the Commission has verified that the five applications fall within the scope of the food enzyme Regulation and contains all the elements required under Chapter II of that Regulation.

1.1.2. Terms of Reference

The European Commission requests the EFSA to carry out the safety assessments on the food enzymes Pullulanase from *Klebsiella pneumoniae* (strain AE-PUL), Pullulanase from *Pullulanibacillus naganoensis* (strain AE-PL), Rhizopuspepsin from *Rhizopus niveus* (strain AE-N), Rennet paste from abomasum of goat

[1] Regulation (EC) No 1332/2008 of the European Parliament and of the Council of 16 December 2008 on Food Enzymes and Amending Council Directive 83/417/EEC, Council Regulation (EC) No 1493/199, Directive 2000/13/EC, Council Directive 2001/112/EC and Regulation (EC) No 258/97. OJ L 354, 31.12.2008, p. 7–15.

[2] Regulation (EC) No 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 354, 31.12.2008, p. 1–6.

(*Capra aegagrus hircus*), sheep (*Ovis aries*) and cattle (*Bos primigenius*), Cellulase from a genetically modified strain of *Trichoderma reesei* (DP-Nzc36) in accordance with Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

1.2. Interpretation of the Terms of Reference

The present scientific opinion addresses the European Commission request to carry out the safety assessment of the food enzyme Pullulanase from *Pullulanibacillus naganoensis* (strain AE-PL).

1.3. Information on existing authorisations and evaluations

The applicant reports that the French, Japanese and Chinese authorities have evaluated and authorised the use of pullulanase from *P. naganoensis* in starch processing.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier supporting the application for authorisation of the food enzyme pullulanase from *P. naganoensis* (strain AE-PL). The food enzyme is intended to be used in the starch processing for the production of glucose syrups.

2.2. Methodologies

The assessment was conducted in line with the principles described in the EFSA 'Guidance on transparency in the scientific aspects of risk assessment' (EFSA, 2009) and following the relevant existing guidances from the EFSA Scientific Committee.

The current 'Guidance on the submission of a dossier for safety evaluation of a food enzyme' (EFSA CEF Panel, 2009) has been followed by the CEF Panel for the evaluation of this application.

3. Assessment

3.1. Technical data

3.1.1. Identity of the food enzyme

IUBMB nomenclature:	Pullulanase
Systematic name:	Pullulan 6-α-glucanohydrolase
Synonyms:	α-dextrin endo-1,6-alpha-glucosidase
IUBMB No:	EC 3.2.1.41
CAS No:	9075-68-7
EINECS No:	232-983-9.

3.1.2. Chemical parameters

The pullulanase produced with the non-genetically *P. naganoensis* (strain AE-PL) is a single polypeptide chain of 958 amino acids. The molecular mass, derived from the amino acid sequence, was calculated to be about 105 kDa. The protein pattern, examined by size-exclusion chromatography, showed three major protein peaks and a number of other minor peaks. However, their identity and molecular mass were not identified.

Data on the chemical parameters of the food enzyme have been provided for three commercial food enzyme batches (Table 1). The average pullulanase activity was 783 pullulanase Units/g (range 727–816 pullulanase Units/g). The presence of other enzyme activities was not reported by the applicant.

The average total organic solids (TOS) content of the three commercial food enzyme batches was 3.1% (w/w); the values ranged from 2.6% to 3.5% (Table 1). The TOS content is a calculated value derived as 100% – % water – % ash – % excipients. The average enzyme activity/TOS ratio of the three commercial enzyme batches was 25.8 Units/mg TOS; the values ranged from 23.3 to 28.0 Units/mg TOS (Table 1; Batches 1–3).

Two additional batches of the food enzyme were used for toxicological studies; the first (Batch 4) for the bacterial reverse mutation (Ames) test and the second (Batch 5) for the remaining studies reported. Both batches showed approximately twice the enzyme activity of the commercial batches (Table 1) but had a similar specific activity when expressed as Units/mg TOS.

Table 1: Compositional data of the food enzyme preparations

Parameter	Unit	Batches				
		1	2	3	4[(c)]	5[(d)]
Pullulanase activity	Units/g batch[(a)]	816	727	807	1,720	1,260
Protein	%	1.8	1.6	1.7	NA[(e)]	NA[(e)]
Ash	%	15.3	14.8	14.9	2.4	3.9
Water	%	68.8	68.5	69.1	90.1	88.8
Total organic solids (TOS)[(b)]	%	3.5	2.6	3.1	7. 5	7.3
Pullulanase activity/mg TOS	Units/mg TOS	23.3	28.0	26.0	22.9	17.4
Excipients	%	12.4	14.1	12.9	–	–

(a): Units/g batch: Pullulanase activity (see Section 3.1.3).
(b): TOS calculated as 100% − % water − % ash − % excipients.
(c): Batch used for the Ames test.
(d): Batch used for the chromosomal aberrations test and the 90-day study.
(e): Not Analysed.

The lead, cadmium and mercury contents of the three commercial batches of the food enzyme were below the limit of detection (LOD: 1–5 μg/kg) and thus well below the specification levels set for food additives (5 mg/kg for lead; 1 mg/kg for cadmium and 1 mg/kg for mercury) (Regulation (EU) No 231/2012[3]). Trace amounts of arsenic were detected in two of the three batches at concentrations which did not give rise to concern (0.003 and 0.032 mg/kg).

Mycotoxins (aflatoxins (B1, B2, G1 and G2), ochratoxin A, sterigmatocystin, T-2 toxin, zearalenone, DON, HT2) were analysed in the three commercial batches and were below the LODs of the applied methodologies.

The food enzyme complies with the microbiological criteria as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006), which stipulate that *Escherichia coli* and *Salmonella* species are absent in 25 g of sample and total coliforms are not more than 30 colony-forming units (CFU) per gram.

The applicant has provided information on the identity of the antifoam agent used. Taking into account the nature and properties of the antifoam agent, the manufacturing process and the quality assurance system implemented by the applicant, the Panel considers its use as of no safety concern.

The Panel considered the compositional data provided for the food enzyme to be sufficient.

3.1.3. Properties of the food enzyme

Pullulanase catalyses the hydrolysis of alpha-1,6 glycosidic linkages in pullulan or starch. Hydrolysis of pullulan results in the production of maltotriose and hydrolysis of starch results in short chains of amylose derived from the amylopectin fraction.

The pullulanase activity is determined based on the hydrolysis of pullulan and is expressed in pullulanase Units/g. One unit of pullulanase activity is defined as the amount of enzyme that liberates reducing sugar equivalent to 1 μmol of glucose per minute under the conditions of the assay (30 min at pH 5 and 40°C).

The temperature/activity profile of the food enzyme was measured from 40°C to 70°C. The pullulanase is active at temperatures below 70°C with an optimum range between 60°C and 65°C at pH 5.0. The optimum pH for activity lies between pH 5.0 and 6.0 at 40°C. Thermostability, determined by pre-incubation, showed that activity is retained at temperatures up to 65°C but lost at higher temperature.

[3] Regulation (EU) No 231/2012 of the European Parliament and of the Council of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83/1, 22.3.2012.

3.1.4. Information on the source material

3.1.4.1. Information related to the taxonomy of microorganism

According to the CEF Guidance (EFSA, 2009, 2014), the certificate of deposit of the strain in a public-validated culture collection should be provided. The applicant deposited the pullulanase production strain *P. naganoensis* only in the Amano internal culture collection. The Panel noted that this would not allow a verification of the strain independently of the company.

The pullulanase-producing bacterium was originally isolated from compost in Japan (Hatayama et al., 2006). Its identification as *P. naganoensis* (http://www.bacterio.net/pullulanibacillus.html) was initially based on nucleotide sequences of 3′-end of the 16S rDNA sequence (150 bp) and the 5′-end of the 16S-23S ITS region (70 bp) (Goto et al., 2000; Hatayama et al., 2006). In addition to the phylogenetic analysis, the chemotaxonomic and physiological characterisation indicated that *P. naganoensis* did not belong to the genus *Bacillus.* The production strain *P. naganoensis* AE-PL was derived from the original isolate through conventional mutagenesis.

Subsequently, the full 16S rDNA gene of the production strain was sequenced and a further phylogenetic analysis was made which confirmed the identity as *P. naganoensis*.

3.1.4.2. Characteristics of the production microorganism

3.1.4.2.1. Phenotypic and genotypic characteristics

Pullulanibacillus naganoensis is a Gram-positive, mesophilic, obligately aerobic, moderately acidophilic, endospore-forming rod, producing a thermostable pullulanase (Tomimura et al., 1990).

According to the applicant, the organism has been used for the production of food enzymes for at least 15 years.

3.1.4.2.2. Production strain toxigenicity, pathogenicity and antimicrobial resistance

Pullulanibacillus naganoensis is currently not included in the list of microorganisms considered suitable for the qualified presumption of safety (QPS) approach (EFSA BIOHAZ Panel, 2017). The limited body of knowledge and published information on this species did not allow EFSA to make a presumption of safety.

The pathogenic potential of the *P. naganoensis* isolate has been tested in BALB/c mice (Tomimura et al., 1990). The bacterium was grown in tryptic soy broth (pH 5.5) at 33°C for 24 h. Tenfold dilutions were introduced into the mice via oral or intraperitoneal routes (eight mice per group). Challenge doses up to 10^7 cells were administered. Animals were observed for 21 days and then examined post-mortem. Immediately following injection, mice challenged intraperitoneally with 10^6 or more cells showed slight to moderate distress, as evidenced by ruffled fur and huddling together of cage occupants. Within 24 h, all mice appeared to recover. No effects were seen in other groups. No abnormalities were seen at necropsy. It was concluded that the *P. naganoensis* isolate was non-pathogenic and non-toxigenic under the test conditions.

No antibacterial activity was detected in the food enzyme.

3.1.5. Manufacturing process

The food enzyme is manufactured in accordance with Regulation (EC) No 852/2004[4], with food safety procedures based on HACCP (Hazard Analysis and Critical Control Points) principles, and in accordance with current good manufacturing practice (GMP).

The food enzyme is produced by a pure culture in a contained, submerged, batch fermentation system with conventional process controls in place. The identity and purity of the culture are checked at each transfer step from frozen vials until the end of fermentation.

After completion of the fermentation, the solid biomass is removed from the fermentation liquor by filtration. The filtrate containing the enzyme is then concentrated by ultrafiltration using membranes capable of retaining the enzyme and allowing much of the low-molecular weight material to pass. Further low-molecular weight material is then removed by diafiltration. Finally, the enzyme concentrate is passed through a final membrane capable of removing any contaminating bacterial cells.

Sorbitol and sodium chloride are added for enzyme stabilisation. The food enzyme is then formulated as a liquid or solid product.

[4] Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. OJ L 226, 25.6.2004, pp. 3–21.

The absence of the production strain in the product was demonstrated using non-selective culturing at 37°C for 2 days. One production batch and three concentrated enzyme stock solutions obtained from commercial production and microfiltered were analysed in triplicate. The final food enzyme preparation was shown to contain less than 10 CFU/g aerobic bacteria.

The Panel considered the information provided on the raw materials and the manufacturing process to be sufficient.

3.1.6. Reaction and fate in food

Pullulanase catalyses the hydrolysis of alpha-1,6 glycosidic linkages in pullulan or starch. Hydrolysis of pullulan results in the production of maltotriose and hydrolysis of starch results in short chains of amylose derived from the amylopectin fraction.

Experimental data on the significant removal (> 99%) of protein in the course of this process have been provided (Documentation provided to EFSA No 5). The Panel considered the evidence as sufficient to conclude that residual amounts of TOS are removed by the purification steps applied during the production of glucose syrups, i.e. filtration, ion exchange chromatography, carbon treatment and crystallisation.

3.1.7. Case of need and intended conditions of use

The food enzyme is intended to be used in starch processing for the production of glucose syrups at the recommended dose of 16–17 mg TOS/kg starch for the production of maltose and glucose syrups which are further used as a food ingredient.

In starch processing for production of glucose syrups, pullulanase is used to optimise the production process. The food enzyme is added during the saccharification step at the beginning of this process.

3.2. Dietary exposure

The Panel considered the evidence provided as sufficient to conclude that the presence of residual amounts of TOS after the purification steps applied during the production of glucose syrups, i.e. filtration, ion exchange chromatography, carbon treatment and crystallisation, is negligible (see Section 3.1.6). Consequently, no exposure was calculated.

3.3. Toxicological data

The test materials used in the toxicological studies are described in Section 3.1.2.

3.3.1. Genotoxicity

3.3.1.1. Bacterial reverse mutation (Ames) test

A bacterial reverse mutation test was performed according to OECD Test Guideline 471 (OECD, 1997a) and following Good Laboratory Practice (GLP) in four strains of *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537) and *E. coli* WP2 *uvrA* in a dose-range finding (DRF) test and a main test in the presence or absence of metabolic activation applying the pre-incubation method. Based on the results obtained in the DRF test, five concentrations were selected for the main test ranging from 9.77 to 5,000 μg/plate (corresponding to 0.74 and 379.5 μg TOS/plate, respectively) without S9-mix and ranging from 39.1 to 5,000 μg/plate (corresponding to 2.7 and 379.5 μg TOS/plate, respectively) with S9-mix. Appropriate positive control chemicals induced significant increases in revertant colony numbers, confirming the sensitivity of the tests and the efficacy of the S9-mix; negative controls (water as vehicle control) were within the historical control ranges. No statistically significant increases in the number of revertant colonies were observed in any tester strain, in the absence or presence of metabolic activation.

The Panel concluded that the food enzyme pullulanase did not induce gene mutations in the bacterial reverse mutation assay under the test conditions employed for this study.

3.3.1.2. *In vitro* chromosomal aberration test

The in vitro chromosome aberration test was carried out according to the OECD Test Guideline 473 (OECD, 1997b) and following GLP. Chinese hamster lung fibroblast (CHL/IU) cells were exposed to the food enzyme pullulanase in a short treatment (6 + 18 h recovery) in the presence and absence of

S9-mix and a continuous treatment for 24 and 48 h without S9-mix in a preliminary cell-growth inhibition test as well as a main chromosome aberration test. Based on the results of the cell-growth inhibition test, the dose levels for the chromosome aberration assay were set at 5,000, 2,500, 1,250 and 625 μg/mL (corresponding to 362.5, 181.3, 90.6 and 45.3 μg TOS/mL, respectively) for both short-term treatments with and without metabolic activation and 24-h continuous treatment. In the 48-h continuous treatment, the dose levels were set at 2,500, 1,250, 625 and 313 μg/mL (corresponding to 181.3, 90.6, 45.3 and 22.7 μg TOS/mL, respectively). Appropriate vehicle (water) and positive controls were used. All positive control compounds induced a statistically significant increase of chromosomal aberration frequency and the system was considered sensitive and valid. Two hundred cells were scored per concentration. In the chromosomal aberration test, no cytotoxicity was observed in most of the experimental points, except the 48-h treatment where more than 50% cell growth inhibition was detected at 2,500 μg/mL and above. No statistically significant increase in the frequency of chromosomal aberrations was observed after treatment with the test article at any concentration analysed.

The Panel concluded that the food enzyme pullulanase did not induce chromosome aberrations under the test conditions employed for this study.

3.3.2. Repeated dose 90-day oral toxicity study in rodents

A repeated dose 90-day oral toxicity study in rodents was performed in accordance with the test guidelines (Notification No. 29, Japanese Ministry of Health and Welfare, 1996) and following GLP.

Four groups of Sprague–Dawley strain specific-pathogen-free rats [Crj:CD(SD)IGS], each comprising 12 male and 12 female rats, received the food enzyme by daily gavage for 91 days at doses of 0, 2.1, 4.3, 8.5 mL/kg body weight (bw) per day (equivalent to 0, 2,190, 4,485 or 8,866 mg/kg bw per day, corresponding to 0, 158.8, 325.2 and 642.8 mg TOS/kg bw per day) (Yamaguchi, 2005).

No treatment-related deaths or changes in clinical signs, body weight, food and water consumption, urinalysis, ophthalmoscopy and macroscopic or microscopic pathology were observed.

In haematology, only significant but slight reductions in prothrombin time were observed in males of the high-dose group and of the activated partial thromboplastin time in males of the medium- and high-dose group. A reduced total protein value was observed in males of the high-dose group. In addition, relative brain weight was found to be significantly increased in the mid-dose males. These effects were minor and were not considered to be of biological relevance.

3.4. Allergenicity

The potential allergenicity of the pullulanase produced with *P. naganoensis* (strain AE-PL) has been assessed by comparing its amino acid sequence with those of known allergens according to the EFSA Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed of the Scientific Panel on Genetically Modified Organisms (EFSA GMO Panel, 2010). Using higher than 35% identity in a window of 80 amino acids as the criterion, no match was found.

Pullulanase from *P. naganoensis* (strain AE-PL) is not described as a potential allergen and no food allergic reactions to this pullulanase have been reported; so, there is no evidence for potential allergenicity of this food enzyme.

According to the information provided, substances or products that may cause allergies or intolerances (Regulation (EU) No 1169/2011[5]) are used as raw materials (soybean meal) in the media fed to the microorganisms. However, the proteins will be digested during the fermentation process and consumed by the microorganisms for cell growth, cell maintenance and production of enzyme protein. In addition, the microbial biomass and fermentation solids will be removed. Therefore, potentially allergenic residues of these foods employed as protein sources are not expected to be present.

Taken together, the CEF Panel considers that there are no indications for food allergic reactions, and therefore, this pullulanase produced with the *P. naganoensis* (strain AE-PL) is not of concern.

[5] Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004.

Conclusions

Based on the removal of residual amounts of TOS from glucose syrups, consumer exposure is not expected. In addition to the safety of the manufacturing process, the compositional and biochemical data lead the Panel to conclude that the food enzyme pullulanase from *P. naganoensis* (strain AE-PL) does not give rise to safety concerns under the intended conditions of use.

Documentation provided to EFSA

1) Dossier 'Pullulanase from *Pullulanibacillus naganoensis* (strain AE-PL)'. February 2015. Submitted by Amano Enzyme Inc.
2) Additional information was received from Amano Enzyme Inc. in November 2015.
3) Additional information was received from Amano Enzyme Inc. in January 2017.
4) Additional information was received from Amano Enzyme Inc. in May 2017.
5) Additional information on 'Food enzyme carry/over in glucose syrups'. February 2017. Provided by the Association of Manufacturers and Formulators of Enzyme Products.

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Abbreviations

CAS	Chemical Abstracts Service
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CFU	colony forming units
CHL	Chinese hamster lung
DON	deoxynivalenol
DRF	dose-range finding
EC	Enzyme Commission
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agriculture Organization
GLP	Good Laboratory Practice
GMO	genetically modified organisms
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis and Critical Control Points
IUBMB	International Union of Biochemistry and Molecular Biology
LOD	limit of detection
OECD	Organisation for Economic Cooperation and Development
QPS	Qualified Presumption of Safety
rDNA	ribosomal deoxyribonucleic acid
TOS	total organic solids
WHO	World Health Organization