β-Fructofuranosidase Enzyme from a Non-Genetically Modified *Aspergillus Fijiensis*

PROCESSING AID APPLICATION

Food Standards Australia
New Zealand

Contents

General Information

1. Format of the Application

A.1.1 Information related to changes to Standard 1.3.1 – Food Additives

This application for an amendment to Standard 1.3.1 and related Schedules is prepared pursuant to Section 3.3.1 – Food Additives of the Food Standards Australia New Zealand Application Handbook (FSANZ, 2019a), which requires the following structured format to assess an application for a new food additive:

A. General information on the application

B. Technical information on the food additive

C. Information on the safety of the food additive

D. Information on dietary exposure to the food additive

The application is presented in this format. At the start of each section (A to D), the information that must be addressed therein is specified in more detail. Additionally, an executive summary for the application is provided as a separate electronic document to this application. The application has been prepared in English and submitted electronically, as required within the Food Standards Australia New Zealand Application Handbook (FSANZ, 2019a)

1.1 Applicant

Meiji Food Materia Co., Ltd., is a manufacturer/marketer of specialty food ingredients, food additives and food processing aids.

Meiji Food Materia Co., Ltd.
2-4-16, Kyobashi, Chuo-ku
Tokyo 104-0031
Japan

1.2 Contact Details

1.3 Purpose of the application

This application seeks to modify Schedule 18 (subsection S18—4(5)) to permit the update of the naming of the microbial source for the production of the processing aid β-fructofuranosidase, used in the production of fructo-oligosaccharides, to add *Aspergillus fijiensis*. The purpose is to add the updated name to the existing name of *Apergillus niger* that is already listed within the Code. No further changes to the Code are requested. This application is made solely on behalf of Meiji Food Materia Co., Ltd., the manufacturer/marketer of the Processing Aid. The Processing Aid β-fructofuranosidase has been permitted in Australia and New Zealand since approval in 2013. The Code currently lists the microbial source as either *Aspergillus niger* or *Saccharomyces cerevisiae.* The subject of this application is therefore to update the Code to add *Aspergillus fijiensis* within Schedule 18*.* Approval of this application would provide clarification for food processors that the enzyme preparation derived from *Aspergillus fijiensis* is identical to that currently laid down in the Code as *Aspergillus niger*. Information provided within the application provides evidence to support the update in the name of the microbial source as well as builds upon the safety previously confirmed and outlined within Application A1055.

1.4 Justification for the Application

A. Costs and Benefits of the application

Currently β-fructofuranosidase, produced from *Apergillus niger* used in the production of fructo-oligosaccharides (FOS) is permitted within the Australia and New Zealand Food Standards Code and supports the marketability of the processing aid within the region. The Processing Aid β-fructofuranosidase has been permitted in Australia and New Zealand since approval in 2013. Approval of this application to add the updated name *Aspergillus fijiensis* will prevent any confusion to current and potential future customers and would maintain the ability of Meiji Food Materia Co., Ltd., to continue business within Australia and New Zealand.

In addition, Meiji Food Materia Co., Ltd., is developing sales in the EU, North America, South America and Asian countries for foods, supplements, infant formula and animal foods. With the approval of the name change, Meiji Food Materia Co., Ltd., wish to further develop the FOS business in the Oceania market for these kinds of products.

B. Impact on international trade

The addition of the updated name *Aspergillus fijiensis* to the current listing of *Aspergillus niger* within the Australia New Zealand Food Standards Code, is to prevent any confusion to current clients and customers as to the permissibility of the enzyme for the production of fructo-oligosaccharides within Australia and New Zealand. The potential impact of not adding the name change could be detrimental to the continued business of Meiji Food Materia Co., Ltd., within Australia/New Zealand.

1.5 Information to Support the Application

Information on the type and identity of the β-fructofuranosidase processing aid, the chemical and physical properties and the manufacturing details along with specifications are provided in section 2 of the application. Information to support the safety of the processing aid is presented in Section 3 and is based upon the fact that β-fructofuranosidase from *Aspergillus niger* is currently approved and accepted within the Australia New Zealand Food Standards Code and the request is solely to update the name change to include *Aspergillus fijiensis.* The safety is further corroborated through both toxicological and pathological studies as well as information related to potential allergenicity of the enzyme and the fact that fructo-oligosaccharides manufactured using the enzyme produced via *Aspergillus fijiensis* has gained worldwide acceptance.

1.6 Assessment Procedure

This application seeks to modify Schedule 18 (subsection S18—4(5)) to Standard 1.3.3 to permit the addition of *Aspergillus fijiensis* to the microbial source list. Based on guidance in the Application Handbook, Meiji Food Materia Co., Ltd., considers the General Procedure to be the appropriate procedure for assessment of the application.

1.7 Confidential Commercial Information (CCI)

Certain (identified) personal, technical and manufacturing information included in the dossier and Annexes 1, 4, 5, 6, 7, 8, 10 and 11 is regarded by the applicant as Confidential Commercial Information (CCI) and is provided in the application strictly on this basis. Non-Confidential versions of the dossier and Annexes are also provided.

Meiji Food Materia Co., Ltd., Meiji consider the information considered to be CCI is either for personal non-disclosure purposes or the result of a significant research and development program/effort and investment by the applicant; it is not in the public domain and is considered

as either proprietary or commercially sensitive. It would be disadvantageous to the applicant if this information were released into the public domain to be obtained by competitors. Versions of the documents/reports containing CCI are provided in both a confidential and redacted format for review. Information considered to be CCI include the statutory declaration (for signature purposes), the detailed manufacturing information and specification results and information regarding safety including toxicological and pathogenicity tests, allergenicity analysis and exposure evaluation.

1.8 Confidential Commercial Information (CCI)

No other confidential information is contained within this application.

1.9 Exclusive Commercial Capturable Benefit (ECCB)

According to Section 8 of the FSANZ Act, this application is not expected to confer Exclusive Capturable Commercial Benefit (ECCB) on the basis that the request is to simply modify Schedule 18 (subsection S18—4(5)) to permit the update of the naming of the microbial source to add *Aspergillus fijiensis* for the production of the processing aid β-fructofuranosidase, used in the production of fructo-oligosaccharides.

1.10 International and Other National Standards

Refer to Section 3.4 for details of International Regulatory authorisations and other National standards. In general, fructo-oligosaccharides produced from β-fructofuranosidase have gained International approval/recognition in Canada, France, Japan the U.S., as well as in Australia and New Zealand.

1.11 Statutory Declaration

A signed Statutory Declaration for Australia is provided as Annex 1. (Confidential)

1.12 Checklist

A completed checklist relating to the information required for submission with this application is provided in Annex 2.

2. Technical information on the processing aid

2.1 Information on the type of processing aid

The enzyme that is the basis of the formal FSANZ application is β-fructofuranosidase. The enzyme β-fructofuranosidase (EC 3.2.1.26) is currently listed within the Code as being derived from either *Aspergillus niger* or *Saccharomyces cerevisiae.* The acceptance for the production of short chain fructo-oligosaccharides (FOS) was outlined by FSANZ following review of Application A1055 in 2013. (http://www.foodstandards.gov.au/code/applications/pages/applicationa1055shor4991.aspx.). Meiji Food Materia Co., Ltd., the provider of the β-fructofuranosidase and the technical information and details of the enzyme outlined within application A1055, now wish to add the more recently identified name of *Aspergillus fijiensis* to the current name *Aspergillus niger* listed in the table of subsection S18—4(5) of Schedule 18 in the Australia New Zealand Food Standards Code (the Code). The reference for supporting the latest classification is Varga *et al* 2011. Both the enzyme and the microbial source are considered identical to that submitted as part of application A1055, the only difference being that the microbial source name has been updated, while the ATCC number (20611™) has remained the same, thereby signifying their identical nature.

2.2 Information on the identity of the processing aid

Common or Usual Name:	β–fructofuranosidase
Trade Name:	Fructofuranosidase (FFCFF)
Chemical Name:	β-D-fructofuranoside fructohydrolase
International Union of Biochemistry and Molecular Biology (IUBMB) Enzyme Nomenclature:	β-fructofuranosidase
IUBMB Number: [Enzyme Commission (EC) Number]	EC 3.2.1.26
Chemical Abstracts Service (CAS) Number:	9001-57-4

The enzyme as outlined within the Code is listed as being derived from either *Aspergillus niger* or *Saccharomyces cerevisiae (non genetically modified).* The proposal is to update the listing to include the source name *Aspergillus fijiensis* (non genetically modified), to those already listed.

As noted within the Code, the source of β-fructofuranosidase was deposited as *Aspergillus niger.* The initial deposit was conducted in association with the filing of a patent and was classified by the American Type Culture Collection (ATCC) as ATCC® 20611™. However, following modern characterisation technology the original strain was identified as *A. japonicus* Saito in 1998 by the ATCC. This strain has now been re-identified based upon gene sequence analyses that was conducted by the ATCC in 2015. The genotypic testing of ATCC® 20611™ included ITS and calmodulin sequencing which showed 100% homology to the species termed *Aspergillus fijiensis* Varga (ATCC® 20611™ (Varga et al 2011) (see Annex 3).

2.3 Information on the chemical and physical properties of the processing aid

As outlined in Application A1055, the β-fructofuranosidase food enzyme is produced from a non-genetically modified microbial source of *Aspergillus.* The naming of the microbial source has changed from *Aspergillus niger,* to *A. japonicus* and more recently to *A. fijiensis.* The molecular weight of the enzyme is approximately 340,000 g/mol (340,000 Da or 340 kDa) as measured by gel filtration (Hirayama *et al.*, 1989; Hayashi *et al*., 1992) and approximately 100,000 g/mol (100,000 Da or 100 kDa) as estimated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). This difference in molecular weight was reported to be due to subunit structures in the food enzyme (Hirayama *et al.*, 1989). The majority of the enzyme preparation is composed of protein and carbohydrate, with the remainder of the product consisting of moisture, fat and ash. The final food enzyme is tested in line with the Joint FAO/WHO Expert Committee on Food Additives (JECFA) requirements for microbially-derived enzyme preparations.

The β-fructofuranosidase is not modified by post-translational processes or technological procedures and is not protein engineered.

The primary enzymatic activity is fructose-transferring activity.

There are no/limited side activities and the stability of the enzyme has been demonstrated *via* real-time and accelerated shelf-life studies.

2.3.1 Reaction and Fate in Food

The use of β-fructofuranosidase as laid down within the Code is for the manufacture of fructo-oligosaccharides (FOS) only, using sucrose as a substrate. No change from the currently accepted use in Australia/New Zealand is requested. The enzyme acts as both an invertase on sucrose molecules and a fructosyltransferase between sucrose molecules and fructofuranosyl-sucrose molecules (*i.e.*, comprising fructose chains with a terminal glucose). Once this reaction is complete, the ingredient undergoes several inactivation and filtration steps to ensure the

complete removal of the enzyme from the final ingredient. Since the enzyme is not present in the final FOS ingredient, the food enzyme will also not be present in the final food.

2.3.2 Proposed Uses in Specific Food Products or Food Categories

The β-fructofuranosidase food enzyme is to be used as a processing aid in line with the currently accepted use in Australia/New Zealand in the production of FOS only, from sucrose. No changes to the currently permitted range of food products is requested. The final FOS material is used as an ingredient in a range of general food products for the purposes of fibre enrichment and/or sugar reduction and for infant formula, foods for infants and supplementary formulated foods for young children.

2.4 Manufacturing process

As outlined in sections 2.1 and 2.2 above, the application request is to update the current listing within the Code to add the source name *Aspergillus fijiensis*, to those already listed. No significant change has been introduced into the manufacturing process since application A1055. *A. fijiensis* ATCC® 20611™ is a proprietary micro-organism which is a member of the *Aspergillus* section *Nigri.* The food enzyme production process is conducted in line with Hazard Analysis Critical Control Point (HACCP) principles. The manufacturing process as previously outlined in Application A1055, involves 2 main stages, (1) fermentation and (2) purification. The initial seed fermentation is conducted in a seed tank and growth is assessed based on CO2 concentration, pH and mycelium volume. The seed is then transferred to the main fermentation tank where the fermentation is scaled up and conducted over several days. The fermentation medium then undergoes a series of filtration steps and the filtered product is then spray-dried. The dried product is then sieved and blended to ensure consistency. Quality control conditions are conducted to ensure consistency in the production process. All raw materials, processing aids and equipment used in the production of β fructofuranosidase are compliant with Japanese Pharmacopoeia and Food Law. Sodium benzoate is added following the main fermentation stage, acting as a preservative, which is followed by a series of filtration steps. Sodium benzoate is a permitted food additive at a maximum permitted level of 1000 mg/kg in food additives as laid down in Schedule 15 of the Code.

Full details of the manufacturing process and raw materials used for the production are provided in Annex 4. Note that this information is proprietary and "Confidential Commercial Information" status is requested.

2.5 Specification for identity and purity

Proposed Chemical and Microbiological Specification

The proposed chemical and microbiological specifications for the β-fructofuranosidase food enzyme are presented in Table 2.1. This table specifies the compositional and purity requirements established by JECFA (2006).

Table 2.1 Proposed Specifications for β-Fructofuranosidase from *Aspergillus fijiensis* ATCC® 20611™

Parameter	Units	Specification	Method of Analysis Reference
Compositional Parameters			
Total Organic Solids	%	NLT 86	Calculation[1]
Minimum Purity Requirements			
Lead	mg/kg	NMT 5	Chapter 6,A, Standard Methods of Analysis in Food Safety Regulation
Salmonella spp	mass/25 g	ND	Chapter 2, Standard Methods of Analysis in Food Safety Regulation
Coliform bacteria	CFU/g	<30	
Escherichia coli	mass/25 g	ND	
Antibacterial Activity	NA	ND	JECFA/FAO Method

CFU = colony forming units; NA = Not Applicable; ND = not detected; NLT = not less than; NMT = not more than
[1] Total Organic Solids = 100% – (A+W+D), where A=%ash, W=%water and D=%diluents and/or other additive and formulation ingredients

Internal specifications and the methods of analysis are provided in Annex 5. (Confidential)

Potential Impurities and Contaminants

As β-Fructofuranosidase is produced from a microbial source, the final enzyme is analysed for impurities and contaminants associated with the filamentous fungi, namely mycotoxins, as previously outlined in Application A1055. The results presented in Table 2.2 demonstrate that levels for these parameters are below the limits of detection for all batches. In line with the Joint Food and Agriculture Organisation/World Health Organisation Expert Committee on Food Additives (JECFA) requirements for enzyme preparations from microbial sources, the enzyme preparation is also tested for microbiological parameters, heavy metals and antibacterial activity. (certificates of analysis for the batches outlined in Table 2.2 below are provided in Annex 6)

No allergens as listed within section 1.2.3—4 in the Code are present within the β-Fructofuranosidase produced using *Aspergillus fijiensis.*

Table 2.2	**Analysis for Potential Impurities and Contaminants of 4 Manufactured Batches of β-Fructofuranosidase**					
Parameter	**Unit**	**Method of Analysis Reference**	**Manufacturing Batch Number**			
			FFCFF-400	**FFCFF-500**	**FFCFF-600[1]**	**FFCFF-700**
Heavy Metals						
Heavy Metals (as Pb)	mg/kg	Sodium sulphide colorimetric method	6	8	7	7
Lead	mg/kg	Chapter 6,A, Standard Methods of Analysis in Food Safety Regulation	NMT 5	NMT 5	NMT 5	NMT 5
Arsenic (as As_2O_3)	mg/kg	1.11, Method 3, Japanese Pharmacopoeia	NMT 1	NMT 1	NMT 1	NMT 1
Cadmium	mg/kg	Atomic absorption spectrometry	0.03	0.03	0.02	0.03
Mercury	mg/kg	Cold vapour atomic absorption spectrometry	ND[2]	ND[2]	ND[2]	ND[2]
Mycotoxins						
Ochratoxin A	µg/kg	HPLC	ND[3]	ND[3]	ND[3]	ND[3]
Aflatoxin B1	µg/kg	Chapter 6,C, Standard Methods of Analysis in Food Safety Regulation	ND[4]	ND[4]	ND[4]	ND[4]
Aflatoxin B2	µg/kg		ND[4]	ND[4]	ND[4]	ND[4]
Aflatoxin G1	µg/kg		ND[4]	ND[4]	ND[4]	ND[4]
Aflatoxin G2	µg/kg		ND[4]	ND[4]	ND[4]	ND[4]
Sterigmatocystin	mg/kg	Chapter 5,II 2 Explanation of Feed Analysis, Japanese Scientific Feeds Association	ND[5]	ND[5]	ND[5]	ND[5]
Microbiological Parameters						
Total viable counts (incl. Yeasts and moulds)	CFU/g	5.02-1, Japanese Pharmacopeia	<1,000	<1,000	<1,000	<1,000
Coliform bacteria	CFU/g	Chapter 2, Standard Methods of Analysis in Food Safety Regulation	<30	<30	<30	<30
Escherichia coli	mass/25 g		ND	ND	ND	ND
Salmonella	mass/25 g		ND	ND	ND	ND
Staphylococcus aureus	mass/g		ND	ND	ND	ND
Anaerobic sulphite-reductors	<30 CFU/g		<30	<30	<30	<30
Viable Moulds Count	mass/0.1 g	Chapter 3, Standard methods of analysis in food safety regulation	Negative	Negative	Negative	Negative
Viable Yeast Count	mass/0.1 g		Negative	Negative	Negative	Negative
Miscellaneous						
Antibacterial Activity	NA	JECFA/FAO Method	ND	ND	ND	ND

CFU = colony forming units; HPLC = high performance liquid chromatography; JECFA = Joint FAO/WHO Expert Committee on Food Additives; NA = Not Applicable; ND = Not Detected; NMT = Not More Than
[1] Batch used for toxicological tests [2] Quantification Limit: 0.01 mg/kg [3] Quantification Limit 0.5 ppb
[4] Quantification Limit: 5 ppb [5] Quantification Limit: 0.5 mg/kg

2.6 Analytical method for detection

Since no β-fructofuranosidase or breakdown or by-products are present in the final food, an analytical method is not required.

3. Information related to the safety of an enzyme processing aid

3.1 General information on the use of the enzyme as a food processing aid in other countries

FOS produced by β-fructofuranosidase from the same microbial source whose name has been updated to *Aspergillus fijiensis* has been accepted worldwide for use in general food and food for infants.

3.2 Information on the potential toxicity of the enzyme processing aid

The β-fructofuranosidase food enzyme has previously been evaluated by FSANZ and considered safe for the production of fructo-oligosaccharides (scFOS), for use in the preparation of infant formula, infant foods and supplementary foods for young children as an alternative to inulin-derived substances as well as general foods (FSANZ, 2013).

The application is not seeking to expand the use of the β-fructofuranosidase food enzyme. The purpose of this application is solely to broaden the current approval to update the name of the microbial source from *Aspergillus niger* to include *Aspergillus fijiensis* within subsection S18—4(5) of Schedule 18 in the Australia New Zealand Food Standards Code.

As noted within the FSANZ Code, the source of β-fructofuranosidase was deposited as *Aspergillus niger* in association with the filing of a patent that was classified by the American Type Culture Collection (ATCC) as ATCC® 20611™. However, following modern characterisation technology the original strain was identified as *A. japonicus* Saito in 1998 by the ATCC. This strain has now been re-identified based upon gene sequence analyses that was conducted by the ATCC in 2015. The genotypic testing of ATCC 20611 included ITS and calmodulin sequencing which showed 100% homology to the species termed *Aspergillus fijiensis* Varga (ATCC® 20611™ (see Annex 3).

While the naming of the strain and the publication authors have changed over the years, the ATCC number has remained consistent throughout the name changes, indicating that these microbial source names are essentially synonyms. On the basis that there have been no changes to the source organism within the production of β-fructofuranosidase and that Meiji do not wish to expand upon the current use of the processing aid, the data used to support the

safety of FOS produced by β-fructofuranosidase-catalysed condensation of sucrose outlined in Application A1055 (FSANZ, 2013), can still be relied upon. http://www.foodstandards.gov.au/code/applications/pages/applicationa1055shor4991.aspx.

With regards to the safety of the enzyme, FSANZ specifically considered the safety of the production organism (*A. niger* ATCC 20611), based upon history of safe use of the enzyme in food production processes and relevant published data on the hazards of the protein and the presence of the production microorganism and/or enzyme in the enzyme preparation and final ingredient. Based on the established history of safe use of various strains of *A. niger* to produce food-grade enzyme preparations under highly controlled conditions, as well as the absence of detectable levels of the production organism in the final food ingredient, FSANZ concluded that the use of *A. niger* ATCC 20611 as a new production organism for β-fructofuranosidase raises no public health and safety issues and therefor considered an acceptable daily intake (ADI) of "not specified" to be appropriate.

Subsequent to the FSANZ application in 2013, toxicological studies on the β fructofuranosidase food enzyme have been conducted. The toxicological tests consisted of 2 *in vitro* genotoxicity tests, including a bacterial reverse mutation test and an *in vitro* micronucleus assay and a 90-day oral toxicity study conducted in rats, the results of which are described below. The original test reports are provided in Annex 7 (Unpublished and Confidential).

3.2.1 Genotoxicity

3.2.1.1 Bacterial Reverse Mutation Test

The mutagenicity of β-fructofuranosidase was evaluated in the bacterial reverse mutation (Ames) test at concentration levels ranging from 156 to 5,000 µg/plate in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and in *Escherichia coli* strain WP2*uvrA* (BoZo Research Center Inc., 2014a [Unpublished, Confidential; see Annex 7]). This study was conducted in compliance with the principles of Good Laboratory Practice (GLP) established by the Organization for Economic Co-Operation and Development (OECD) (OECD, 1998a) and the Japanese Ministry of Health and Welfare Ordinance No. 21 and 114 (MHLW, 1997, 2008), and performed in accordance to OECD Test Guideline No. 471 (OECD, 1997). Water was used as the negative control and the test vehicle for all strains. The positive controls consisted of 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide , sodium azide, and 2-methoxy-6-chloro-9-[3-(2-chloroethyl)aminopropylamino] acridine·2HCl for the assays conducted without S9 metabolic activation; whereas the positive controls for the assays conducted with S9 metabolic activation included 2-aminoanthracene and benzo[*a*]pyrene.

The results of the bacterial reverse mutation (Ames) test confirmed that β-fructofuranosidase is not mutagenic.

3.1.1.2 In Vitro Mammalian Cell Micronucleus Test

An *in vitro* micronucleus test was also conducted using cultured mouse lymphoma cells (L5178Y $tk^{+/-}$ –clone 3.7.2c) to evaluate the potential for β-fructofuranosidase to induce genotoxicity in mammalian cells [BoZo Research Center Inc., 2014b (Unpublished, Confidential: see Annex 7)]. This study was conducted under GLP principles established by the OECD (1998a) and the Japanese Ministry of Health and Welfare Ordinance No. 21 and 114 (MHLW, 1997, 2008). This study was also performed in accordance to the OECD Test Guideline No. 487 (OECD, 2010) and the Japanese Ministry of Health, Labour, and Welfare Guidance for the Evaluation of Genetic Toxicity Studies of Pharmaceuticals (MHLW, 2012). L5178Y cells were used at passage number 13 for the dose selection test and passage number 20 for the main test, and subcultivation was performed every 1 to 4 days. β-Fructofuranosidase was tested under several experimental conditions including a short-term culture of 3 hours with and without metabolic activation and a continuous 24-hour culture in the absence of S9 metabolic activation. Water was used as the test vehicle and also served as a negative control. The positive controls for the short-term treatment with and without S9 metabolic activation were cyclophosphamide and colchicine, respectively, while the positive control for the continuous treatment was mitomycin C. The presence of micronuclei was microscopically assessed for at least 1,000 cells per slide and was performed in duplicate for each concentration level in a blinded manner.

The results demonstrate that β-fructofuranosidase is not genotoxic in the *in vitro* micronucleus test.

3.2.2 Subchronic Toxicity

The toxicity of β-fructofuranosidase was assessed in a subchronic oral toxicity study conducted in rats [BoZo Research Center Inc., 2014c (Unpublished, Confidential; see Annex 7)]. This study was performed in accordance to OECD Test Guideline No. 408 (OECD, 1998b) and was conducted in compliance with the principles of GLP established by the OECD (1998a) and the Japanese Ministry of Health and Welfare Ordinance No. 21 and 114 (MHLW, 1997, 2008). At 6 weeks of age, Sprague-Dawley SPF rats (10/sex/group) were randomised into 4 groups and orally administered β-fructofuranosidase at dose levels of 0, 100 (low-dose), 300 (mid-dose), or 1,000 (high-dose) mg/kg body weight/day for 91 days. β-Fructofuranosidase was given to rats by oral gavage at volumes of 5 mL/kg body weight and distilled water was used as the vehicle control. Throughout the study period, rats were monitored for clinical observations 3 times daily including external appearance, nutritional condition, posture, behaviour and appearance of excrement. More detailed clinical observations were measured weekly including home case, in-the-hand, and open field observations. Manipulative tests including auditory and approach response, contact reaction, pain response, papillary reflex, air-righting reflex, and landing foot splay and measurement of grip strength of the forelimbs and hindlimbs and motor activity were

conducted in Week 12. Body weight was measured before the start of the study and on Days 1, 4 and 7 and thereafter twice weekly. Food consumption was assessed prior to dosing and on Days 1 and 7 and thereafter once weekly. Ophthalmology and urinalysis were performed before the start of β-fructofuranosidase administration and once during Week 13. Water intake for the period of 1 day was also measured at the time of urinalysis. On the day following the final administration of β-fructofuranosidase, blood was collected for standard haematological and biochemistry analysis prior to necropsy and histopathological examination of various organs and tissues.

On the basis of the results from the study, the NOAEL for β-fructofuranosidase in both male and female rats was determined to be 1,000 mg/kg body weight/day, the highest dose tested. This is equivalent to 920 mg/kg body weight/day when expressed as TOS and 108 mg/kg body weight/day when expressed as protein.

Overall, the safety studies conducted with the β fructofuranosidase food enzyme, report no concerns regarding mutagenicity or systemic toxicity and corroborate the safety evaluation conclusion previously determined by FSANZ following the evaluation of Application A1055.

3.3 Information on the potential allergenicity of the enzyme processing aid

In order to determine whether there are any concerns regarding the allergenicity of the β-fructofuranosidase food enzyme, an amino acid sequence homology search was conducted of the amino acid sequence of β-fructofuranosidase using the Food Allergy Research and Resource Program (FARRP) AllergenOnline Database (version 15.0; available at http://www.allergenonline.org/; updated January 12, 2015) maintained by the Food Allergy Research and Resource Program of the University of Nebraska (FARRP, 2015). This database contains a comprehensive list of putative allergenic proteins developed *via* a peer-reviewed process for the purpose of evaluating food safety. The aim of a sequence homology comparison is to determine the extent to which a substance is similar in structure to known allergens (WHO/FAO, 2009).

A full-length alignment search of the amino acid sequence was conducted in the AllergenOnline database using default settings (E-value cut-off[1] = 1; maximum alignments = 20). A positive match was identified with 'peanut agglutinin precursor [Arachis hypogaea]'. The percent identity (PID) of the amino acid sequence was 25% and the E-value was 0.84, see Annex 8 for the search results. Cross-reactivity of a protein with a known allergen is rare when the primary

[1] E-value (expectation value) is a calculated value that reflects the degree of similarity of the query protein to its corresponding matches; The size of the E-value is inversely related to the similarity of the 2 proteins, meaning a very low E-value (*e.g.,* 10e-30) indicates a high degree of similarity, while a value of 1 or higher indicates that the protein are not likely to be related in evolution of structure

amino acid sequence similarity is less than 50% throughout the length of the protein (Aalberse, 2000). Thus, this was considered to be a poor match. Furthermore, the E-value (expectation value) was close to 1, indicating a low degree of similarity between the 2 proteins. The potential for cross-reactivity between the β-fructofuranosidase food enzyme and peanut agglutinin precursor [Arachis hypogaea] is therefore considered low.

A second homology search was conducted according to the approach outlined in the FAO/WHO and the Codex Alimentarius Commission (FAO/WHO, 2001; WHO/FAO, 2009), whereby the AllergenOnline database was searched using a sliding window of 80-amino acid sequences. Significant homology is defined as an identity match of greater than 35%, and in such instances, cross-reactivity of the enzyme with a known allergen must be considered a possibility (FAO/WHO, 2001). No sequence similarities were identified between the amino acid sequences and any know allergens using this search strategy (see Annex 8; Unpublished Confidential).

Based on the outcome of these two searches, no evidence exists that would indicate that the β-fructofuranosidase food enzyme would cross-react with known allergens. Furthermore, as described in Section 2.4 above and Annex 4, a number of steps are incorporated into the FOS manufacturing process to ensure inactivation and removal of the enzyme from the final ingredient. The potential for an allergenic reaction is thereby further reduced.

3.4 Safety assessment reports prepared by international agencies or other national government agencies, if available

In addition to the safety of the β-fructofuranosidase food enzyme as determined by FSANZ in their evaluation of short-chain fructo-oligosaccharides (scFOS) back in 2013, a number of additional authorisations currently exist for the enzyme (see table 3.1). Recently, Meiji has begun the process of updating the authorisations of the β-fructofuranosidase food enzyme to take in to account the change in name of the microbial source to include *Aspergillus fijiensis*. Updated applications have been made to the Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail (ANSES)] within France and to Health Canada. Both regulatory Authorities have updated their regulations to include the name change. In addition, an application has been filed with the European Food safety Authority (EFSA), to comply with Regulation (EC) No. 1331/2008 of the European Parliament and Council of 16 December 2008. This application is currently under review.

From a historical perspective, BEGHIN MEIJI submitted an initial application for the use of β-fructofuranosidase from *A. niger* ATCC 20611[2] as a processing aid to the French authorities in November 1989. This dossier was evaluated on behalf of the French agency Conseil Supérieur d'Hygiène Publique (CSHP) [replaced by Agence Française de Sécurité Sanitaire des Aliments (AFSSA); now ANSES and considered not to present a consumer safety concern. The enzyme

[2] As noted in Section 3.2.1, prior to 1997, the production strain was categorised as *A. niger*.

was initially authorised for use as a processing aid in the manufacture of foodstuffs and food ingredients in 1993 under *Arrêté du 27 août 1993 modifiant l'arrêté du 05 septembre 1989 relatif à l'emploi de préparations enzymatiques dans la fabrication de certaines denrées et boissons destinées à l'alimentation humaine*. This legislation was replaced by *Arrêté du 19 octobre 2006 relatif à l'emploi d'auxiliaires technologiques dans la fabrication de certaines denrées alimentaires* under which the enzyme is currently listed in Annexe I-C (JORF, 2006). Recently, the ANSES reported in a letter to Meiji that that the production strain of this enzyme will be modified in the annex of the order of October 19, 2006. This enzyme will now be referred to as "β-fructofuranosidase from *Aspergillus niger* (synonyms *Aspergillus fijiensis, Aspergillus japonicus*) (ATCC 20611)". The ANSES based their decision on the fact that the production strain was identified again in 2015 from gene sequence analyses conducted by the American Type Culture Collection (ATCC) (Annex 3). This identification led to a 100% match with the *Aspergillus fijiensis* Varga species (ATCC 20611) (ANSES 2019, see Annex 9).

FOS, produced using β-fructofuranosidase from *A. niger* ATCC 20611, was successfully notified as Generally Recognised as Safe (GRAS) to the FDA in 2000 in a range of foodstuffs, with a subsequent extension of use in 2007 (U.S. FDA, 2000, 2007). As part of this assessment, the use of the enzyme was considered. The FDA had no questions in either instance regarding the applicant's conclusion that FOS, produced using β-fructofuranosidase, is GRAS under the intended conditions of use.

Additionally, invertase (β-fructofuranosidase) from *A.fijiensis*[3] has also been approved in the production of FOS from sucrose (Health Canada, 2020). The List of Permitted Food Enzymes was updated to include *A. fijiensis* as a permitted source organism for invertase. Invertase sourced from *A. fijiensis* is therefore legally enabled for use in sucrose used in the production of fructooligosaccharides, at a maximum level of Good Manufacturing Practice. (https://www.canada.ca/en/health-canada/services/food-nutrition/public-involvement-partnerships/modification-permitted-food-enzymes-aspergillus-fijiensis.html)

In Japan, the β-fructofuranosidase food enzyme is currently classified as a food additive. The entry was listed as an existing food additive 'No. 33, Invertase' (MHLW, 2014a), with no official specification established under the 8th Japanese Specifications and Standards of Food Additives. The 9th Japanese Specifications and Standards of Food Additives, however re-classified the enzyme as 'fructosyl transferase', the definition of which is '*an enzyme that transfers the fructosyl group of sugars and is obtained from mold cultures (Aspergillus genus and Penicillium roqueforti only).*' (MHLW, 2014b).

[3] As described in Section 3.2.1, the production strain was previously classified as *A. niger* and *A. japonicas* but has more recently been identified as *A. fijiensis*

Table 3.1 Existing Authorisations and Evaluations for β-Fructofuranosidase			
Jurisdiction	**Evaluating/Authoritative Body**	**Permitted Uses**	**Reference**
France	Conseil Supérieur d'Hygiène Publique de France[1]	Production of FOS	Arrêté du 19 octobre 2006 relatif à l'emploi d'auxiliaires technologiques dans la fabrication de certaines denrées alimentaires[2] (JORF, 2006). Updated in 2019.
Australia and New Zealand	Food Standards Australia New Zealand (FSANZ)	Production of short chain FOS	Application A1055 (FSANZ, 2013a)
United States	Food and Drug Administration (FDA)	Production of FOS	GRN 000044 (U.S. FDA, 2000, 2007)
Canada	Health Canada	Production of FOS	Health Canada, 2020
Japan	Ministry of Health and Welfare	Foods and food additives	9th Japanese Standards of Food Additives (MHLW, 2014b)

FOS = fructo-oligosaccharides

[1] Competent authority in France prior to ANSES and AFSSA

[2] Formerly Arrêté du 27 août 1993

4. Additional information related to the safety of an enzyme processing aid derived from a microorganism

4.1 Information on the source microorganism

The production microorganism is non-genetically modified, with a recently updated name of *A. fijiensis* ATCC® 20611™. This is a proprietary micro-organism. The strain was originally classified as *A. niger* until November 4, 1997, when the American Type Culture Collection (ATCC) reclassified it as *A. japonicus* based on its morphology (ATCC, 2013; Annex 3). More recently, sequence analyses on the calmodulin and beta-tubulin genes have identified the ATCC® 20611™ strain as belonging to *A. fijiensis* (Annex 3). The current taxonomic classification of *A. fijiensis* ATCC® 20611™ is presented in Table 4.1.

Table 4.1	Taxonomic Classification of *Aspergillus fijiensis* ATCC® 20611™
Taxonomy	**Taxonomic Assignment**
Kingdom	Fungi
Phylum	Ascomycota
Class	Eurotiomycetes
Order	Eurotiales
Family	Trichocomaceae
Genus	*Aspergillus*
Species	*Aspergillus fijiensis*
Strain	*Aspergillus fijiensis* ATCC® 20611™

A. fijiensis is a member of the *Aspergillus* section *Nigri* (the black Aspergilli), which includes 19 other species: *A. niger*, *A. foetidus*, *A. tubingensis*, *A. aculeatus*, *A. brasiliensis*, *A. carbonarius*, *A. costaricaensis*, *A. eucalypticola*, *A. ellipticus*, *A. heteromorphus*, *A. homomorphus*, *A. ibericus*, *A. indologenus, A. japonicas, A. lacticoffeatus*, *A. neoniger, A. piperis*, *A. sclerotiniger*, and *A. vadensis* (EFSA, 2009; Varga *et al.*, 2011).

4.2 Information on the pathogenicity and toxicity of the source microorganism

Production Strain Pathogenicity, Toxigenicity and Antimicrobial Resistance

As outlined within the FSANZ approval report for Application A1055, β-fructofuranosidase (EC 3.2.1.26) was accepted as a safe enzyme and was considered to have been approved internationally. The opinion also highlighted that *A. niger* is recognised internationally as a safe organism, suitable for the manufacture of enzyme preparations. This viewpoint was corroborated in a recent pathogenicity study that was conducted with *A. fijiensis* ATCC® 20611™ in mice [Hashima Laboratory; Nihon Bioresearch Inc 1999 (unpublished, Confidential; see Annex 10)]. The results confirmed that the enzyme producing microorganism was non-pathogenic.

While a search of the literature found no published studies addressing the pathogenic or toxigenic potential of the latest name change *A. fijiensis,* presumably on the basis of the reclassification, a number of studies looking at the assessment of *A. japonicus* were obtained. These data indicated some contradictory findings regarding ochratoxin A production among a number of strains of *A. japonicus*, with some research presenting positive results (Dalcero *et al.*, 2002; Battilani *et al*., 2003; Sparado *et al*., 2012) and other reporting non-detected results (Téren *et al.*, 1996; Samson *et al*., 2004; El Khoury *et al.*, 2008; Frisvad et al, 2011; Kizis *et al.*, 2014). *Aspergillus japonicus* also tested negative for a range of other extrolites, including aflatoxins, fumonisin B2, sclerotia, pyranonigrins, naphtho-γ-pyrones, asperazine, secalonic

acid, antafumicin, aflavinines, Corymbiferan lactones, and kotanins and neoxaline (Samson *et al.*, 2004; El Khoury *et al.*, 2008; Susca *et al.*, 2010). The latter (neoxaline) had previously been reported as a positive extrolite for *A. fijiensis* Fg-551 (Hirano *et al.*, 1979).

Overall, while the data confirm the lack of pathogenicity and toxicity of the microbial source, Meiji continue to monitor the safety of the source organism through the analysis of secondary metabolites and therefore every batch of the enzyme preparation is tested for a range of mycotoxins, including ochratoxin A. As outlined in table 2.2 there are no detectable levels of mycotoxins within the β-fructofuranosidase food enzyme. Furthermore, in line with JECFA specifications, the enzyme is tested for antibacterial resistance.

4.3 Information on the genetic stability of the source organism

The production strain has been deposited with the ATCC as well as with the National Institute of Advanced Industrial Science and Technology Patent Microorganisms Depositary (Reference: FERM P-5886). The strain is stored in an L-tube which is stored between 1 and 10°C. Following incubation on an agar medium, spores are stored in a sterilised medium (20% skimmed milk). The resulting spore suspension is filtered through sterilised absorbent cotton and dispensed in aliquots of 0.2 mL into glass L-tubes and freeze-dried. After freeze-drying, the container is sealed and refrigerated between 1 and 10°C. This L-tube is used as a starter material for every production batch of the food enzyme. The final enzyme produced is tested to internationally recognised (*i.e.*, JECFA) and internal specifications to ensure that all aspects of the enzyme are consistent in terms of composition, activity and toxins, demonstrating the consistency of the production strain.

5. Information related to the dietary exposure to the processing aid

5.1 A list of foods or food groups likely to contain the processing aid or its metabolites

The β-fructofuranosidase food enzyme is used as a processing aid in the production of the FOS ingredient only and is not added directly to food. As per the Approval report for Application A1055, FOS is permitted for use in general foods as well as infant formula products, foods for infants and supplementary formulated foods for young children. As noted previously, the manufacturing process for the FOS ingredient incorporates several inactivation and filtration steps to ensure the complete removal of the enzyme from the final ingredient. As a consequence, even though the potential carry-over of the β-fructofuranosidase food enzyme into foods is extremely unlikely a theoretical maximum potential level of the enzyme in foods was calculated using the Budget method, so as to determine a Theoretical Maximum Daily

Intake (TMDI). The Budget method for determining exposure is based on conservative assumptions regarding physiological requirements for energy from food and the energy density of food rather than on food consumption survey data. The Budget Method was originally developed for determining food additive use limits and is known to result in conservative estimations of the daily intake.

5.2 The levels of residues of the processing aid or its metabolites for each food or food group

While not present in the final food products the exposure assessment was conducted from a maximum theoretical carry over of the β-fructofuranosidase processing aid (Annex 11 Confidential). The calculated maximum potential level of the food enzyme in the production of FOS from sucrose based on the two production processes (with or without immobilisation; See Annex 4) are considered to be highly conservative based upon the following factors:

1. The minimum ratio of sucrose substrate to FOS ingredient has been assumed for both processes; in reality, a higher ratio typically exists, which would reduce the calculated level of the enzyme present in the final FOS ingredient;
2. The lowest enzyme activity as specified in the food enzyme specification has been assumed (i.e., 10,000,000 units/g);
3. The minimum TOS (%) content as specified in the food enzyme (i.e. 86%) has been assumed.
4. It is assumed that 100% of the enzyme is released into the FOS product;

The highest calculated amount of the food enzyme that was assumed to be present in the FOS product was determined to be 27.73 mg TOS/kg FOS. This value was subsequently used for the purposes of calculating the TMDI using the budget method. Therefore, this assessment assumes that the entire enzyme preparation added during the manufacturing process ends up in the final ingredient, whereas in reality there are NO measurable enzyme levels within the final FOS ingredient.

The budget method is routinely utilised as a conservative screening method to assess potential dietary exposure. This methodology was used for the β-fructofuranosidase food enzyme for adults and also for young children based on the inclusion of FOS in foods intended for these age groups. For adults, this was assessed for a standard body weight of 70 kg, whereas a default weight of 12 kg has been recommended for a toddler aged 12 to 36 months. This approach assumes that there is a maximum physiological amount of foods and beverages which can be consumed daily, that 25%[4] of consumed solid foods and non-milk beverages would contain FOS as an ingredient, and that this FOS is manufactured using the *Aspergillus*

[4] Based on the assumptions of the FAO/WHO report, 12.5% of solid foods are assumed to contain the enzyme, however this should be increased to 25% in the case of enzymes used in a wide range of food categories

fijiensis food enzyme (FAO/WHO, 2009). For young children, the method was adapted as per the recommendations of the FAO/WHO report[5], namely by assuming that 100% of beverages consumed contain the enzyme of interest. This results in an estimate of the Theoretical Maximum Daily Intake (TMDI) of the enzyme. This method incorporates the following assumptions:

- Level of Consumption of Foods and Non-Milk Beverages

  The FAO/WHO report specifies the standard values for food and non-milk beverage intakes at 0.05 kg/kg body weight/day for solid foods and 0.1 L/kg body weight/day for non-milk beverages. Using a body weight for adults of 70 kg, this is equivalent to 3.5 kg of solid food and 7 L of non-milk beverages per person per day. Based on the body weight for a toddler (12 kg), this is equivalent to 0.6 kg of solid food and 1.2 L of beverages.

- Level of Presence of Food Enzyme in Foods and Non-Milk Beverages

  The maximum theoretical level of the β-fructofuranosidase food enzyme assumed to be present in FOS was calculated at 27.73 mg TOS/kg. The maximum recommended dosage of FOS in any food or beverage has been determined to be 50% [e.g., jams, marmalades and confectionery], although this is much higher than for foods in general. Based on this approach, the maximum potential level of β-fructofuranosidase in foods and beverages is equivalent to 13.87 mg β-fructofuranosidase per kg of solid food or per litre of non-milk beverage. Among foods intended for young children, the maximum use level of FOS was 6%, this is equivalent to 1.66 mg TOS/kg food.

- Proportion of Solid Foods and Non-Milk Beverages That May Contain Enzyme

  According to the Budget Method, a standard proportion of all solid foods and non-milk beverages (25%) are assumed to contain the food ingredient (*i.e.*, FOS) which is manufactured using the food enzyme (FAO/WHO, 2009). This assumes that a typical adult weighing 70 kg consumes 0.88 kg of solid food and 1.75 L of non-milk beverages containing FOS produced using the β-fructofuranosidase food enzyme. The FAO/WHO report specifies that when assessing intakes for children, the proportion of beverages that may contain the compound of interest should be increased to 100%, thus 1.2 L of beverages containing the enzyme are consumed daily, along with 0.15 kg of solid foods.

[5] http://whqlibdoc.who.int/ehc/WHO_EHC_240_9_eng_Chapter6.pdf (FAO/WHO, 2009)

Theoretical Maximum Daily Intake of the Enzyme

Based on conservative estimates of exposure calculated using the Budget method, the TMDI of β-fructofuranosidase by adults from all foods and beverages was estimated to be 0.52 mg TOS/kg body weight/day. Among young children, this was calculated at 0.02 mg TOS/kg body weight/day (Table 5.2).

Table 5.2 TMDI of β-Fructofuranosidase by Adults and Young Children Based on the Use of FOS in Solid Foods and Non-Milk Beverage

Products	A Consumption of Solid Foods & Non-Milk Beverages (kg/kg bw/d)	B Max Level of FOS in Solids Foods & Non-Milk Beverages (g/kg)	C Consumption of FOS (g/kg bw/d)[1]	D Proportion of Solid Foods & Non-Milk Beverages containing Enzyme (%)	E Consumption of FOS containing Enzyme (g/kg bw/d)	F Max Amount of Enzyme in FOS (mg TOS/kg)	Total Exposure to Enzyme[2] (mg TOS/kg bw/d)
Adults							
Solid Foods	0.05	500	25	25	6.25	27.73	0.17
Non-Milk Beverages	0.1	500	50	25	12.5	27.73	0.35
Total SF&NMB							0.52
Young Children							
Solid Foods	0.05	6	0.3	25	0.08	27.73	0.002
Non-Milk Beverages	0.1	6	0.6	100	0.6	27.73	0.02
Total SF&NMB							0.02

bw = body weight; d=day; FOS=fructo-oligosaccharide; TOS = total organic solids
[1] Calculation: (A)*(B)
[2] Calculation: (E/1000)*(F)

Conclusion on Dietary Exposure Assessment

The TMDI calculated for adults using budget method assumptions for food and beverage intake was 0.52 mg TOS/kg body weight/day. Among young children, exposure was calculated to be 0.02 mg/kg body weight/day.

It is important to note that these assessments assume that the entire enzyme preparation added during the manufacture of FOS remains in the final ingredient, using conservative assumptions regarding the level which may theoretically be present in food. As described previously a number of steps are included in the manufacture of FOS considering either the non-immobilised or immobilised process to ensure complete removal of the enzyme. Thus, all results presented in this section are considered to be a worst-case scenario that was conducted to corroborate the lack of any potential safety concerns following a maximum potential exposure scenario of β-fructofuranosidase.

The calculated FOS consumption levels outlined in Table 5.2 (25 to 50 g/kg body weight/day in adults and 0.6 g/kg body weight/day in young children) are also considered to be considerably higher than would realistically be expected to be consumed on a daily basis.

Toxicological studies were performed using a representative batch of β fructofuranosidase. The toxicological tests consisted of 2 *in vitro* genotoxicity tests and a 90-day toxicity study conducted in rats. The food enzyme was demonstrated not to be mutagenic or genotoxic and a no-observed-adverse-effect level (NOAEL) of 1,000 mg/kg body weight/day (the highest dose tested) equivalent to 920 mg/kg body weight/day when expressed as TOS was determined. When compared to the maximum exposure to the food enzyme based on the very conservative assumptions considered in the dietary exposure assessment using the Budget method, the margin of exposure was determined to be extremely high (1,769 to 30,667) and therefore, the processing aid enzyme β-fructofuranosidase is not expected to present any issues for human safety.

5.3 For foods or food groups not currently listed in the most recent Australian or New Zealand National Nutrition Surveys (NNSs), information on the likely level of consumption

The use of the β-fructofuranosidase enzyme will not change from the currently permitted uses that were accepted as part of the evaluation following Application A1055 and therefore is not expected to be used in the production of any foods or food groups that are currently not listed in NNSs. It should be further noted that an extremely conservative method of exposure was used to determine the potential intake of the processing aid. The considerable margin of exposure that was calculated was therefore deemed large enough to cover any minor changes in food use that may occur in the future.

5.4 The percentage of the food group in which the processing aid is likely to be found or the percentage of the market likely to use the processing aid

As stated above, it is extremely unlikely the processing aid is carried over into the food ingredient and therefore the final food product to which the ingredient is added.

5.5 Information relating to the levels of residues in foods in other countries

As stated above no residues are expected and even if there was some minor carry over the levels would be covered by the extremely high margins of safety determined from the safety data provided.

5.6 For foods where consumption has changed in recent years, information on likely current food consumption

The current food uses of FOS has not changed in recent years from those permitted within the approval following Application A1055.

6. References

Aalberse RC (2000). Structural biology of allergens. Journal of Allergy and Clinical Immunology, 106, 228-238.

ATCC (2013). Product sheets *Aspergillus japonicas*, 20611. American Type Culture Collection (ATCC), Manassas, VA. https://www.lgcstandards-atcc.org/Products/All/20611.aspx?geo_country=gb&slp=1#generalinformation.

Battilani P, Pietri A, Bertuzzi AT, Languasco L, Giorni P, Kozakiewicz Z (2003). Occurrence of ochratoxin A-producing fungi in grapes grown in Italy. Journal of Food Protection, 66, 633–636.

BoZo Research Center Inc. (2014a). A bacterial reverse mutation test of β-fructofuranosidase: final report (English version) [Confidential]. Study No. T-1559. Prepared by BoZo Research Center Inc., Shizuoka & Tokyo, Japan for Meiji Co., Ltd., Kanagawa, Japan.

BoZo Research Center Inc. (2014b). An *in vitro* micronucleus study in cultured mammalian cells treated with β-fructofuranosidase: final report (English version) [Confidential]. Study No. T-G109. Prepared by BoZo Research Center Inc., Shizuoka & Tokyo, Japan for Meiji Co., Ltd., Kanagawa, Japan.

BoZo Research Center Inc. (2014c). 1 90-day oral gavage toxicity study of β-fructofuranosidase in rats: final report (English version) [Confidential]. Study No. B-7537. Prepared by BoZo Research Center Inc., Shizuoka & Tokyo, Japan for Meiji Co., Ltd., Kanagawa, Japan.

Dalcero A, Magnoli C, Hallak C, Chiacchiera SM, Palacio G, Rosa CAR (2002). Detection of ochratoxin A in animal feeds and capacity to produce this mycotoxin by *Aspergillus* section *Nigri* in Argentina. Food Additives and Contaminants, 19, 1065-1072.

EFSA (2009). Scientific Opinion on the maintenance of the list of QPS microorganisms intentionally added to food or feed (2009 update) (EFSA Panel on Biological Hazards/BIOHAZ) (Question no: EFSA-Q-2009-00459, adopted: 10 December 2009 by the European Food Safety Authority). EFSA Journal, 7, 1431 [92 pp] doi:10.2903/j.efsa.2009.1431. http://www.efsa.europa.eu/en/efsajournal/pub/1431.htm.

El Khoury A, Rizk T, Lteif R, Azouri H, Delia ML, Lebrihi A (2008). Fungal contamination and Aflatoxin B1 and Ochratoxin A in Lebanese wine-grapes and musts. Food and Chemical Toxicology, 46, 2244-2250.

FAO/WHO (2001). Evaluation of allergenicity of genetically modified foods. Report of a Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology, 22-25 January 2001. Food and Agriculture Organization of the United Nations, Rome / World Health Organization (WHO), Geneva. ftp://ftp.fao.org/es/esn/food/allergygm.pdf.

FAO/WHO (2009). Chapter 6: Dietary exposure assessment of chemicals in food. In: Principles and methods for the risk assessment of chemicals in food. Environmental Health Criteria, no 240. World Health Organization (WHO), International Programme on Chemical Safety (IPCS), Geneva, Switz.. http://whqlibdoc.who.int/ehc/WHO_EHC_240_9_eng_Chapter6.pdf.

FARRP (2015). AllergenOnline Version 15: Home of the FARRP Allergen Protein Database. University of Nebraska-Lincoln, Food Allergy Research and Resource Program (FARRP), Lincoln, NB. http://farrp.unl.edu/ [Updated 12 January, 2015].

Frisvad JC, Larsen TO, Thrane U, Meijer M, Varga J, Samson RA, Nielsen KF (2011). Fumonisin and ochratoxin production in industrial *Aspergillus niger* strains. PLoS ONE, 6, e23496. doi:10.1371/journal.pone.0023496.

FSANZ (2013a). Application A1055 - short-chain fructo-oligosaccharides. to amend: Australia New Zealand Food Standards Code. Food Standards Australia New Zealand (FSANZ), Canberra, Australia / Wellington, New Zealand. http://www.foodstandards.gov.au/code/applications/pages/applicationa1055shor4991.aspx.

Hayashi S, Matsuzaki K, Takasaki Y, Ueno H, Imada K (1992). Purification and properties of β-fructofuranosidase from *Aspergillus japonicus*. World Journal of Microbiology and Biotechnology, 8, 276-279.

Health Canada (2020). List of permitted food enzymes. (lists of permitted food additives). Health Canada, Ottawa, ON. https://www.canada.ca/en/health-canada/services/food-nutrition/food-safety/food-additives/lists-permitted/5-enzymes.html [Date Modified: 2020-04-15].

Hirano A, Iwai Y, Masuma R, Tei K, Omura S (1979). Neoxaline, a new alkaloid produced by *Aspergillus japonicas*: production, isolation and properties. The Journal of Antibiotics, 32, 781-785.

Hirayama M, Sumi N, Hidaka H (1989). Purification and properties of a fructooligosaccharide-producing β-fructofuranosidase from *Aspergillus niger* ATCC 20611. Agricultural and Biological Chemistry, 53, 667-673.

IUBMB (2014). IUBMB enzyme nomenclature. EC 3.2.1.26. accepted name: β-fructofuranosidase. In: Enzyme nomenclature. recommendations of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology on the nomenclature and classification of enzymes by the reactions they catalyse [website]. Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB). Queen Mary University of London, School of Biological and Chemical Sciences, London, Engl. http://www.chem.qmul.ac.uk/iubmb/enzyme/EC3/2/1/26.html [Last update 14 October, 2014].

JECFA (2006). General specifications and considerations for enzyme preparation used in food processing [prepared by the Committee at its sixty-seventh meeting (2006)]. In: Combined compendium of food additive specifications [online edition]. General specifications for enzymes analytical methods, volume 4: analytical methods, test procedures and laboratory solutions used by and referenced in the food specifications. 1st to 67th JECFA Meetings, 1965-2006. FAO JECFA Monographs 3. Food and Agriculture Organization of the United Nations (FAO), Rome / Joint FAO/WHO Expert Committee on Food Additives (JECFA), Geneva. http://www.fao.org/ag/agn/jecfa-additives/docs/enzymes_en.htm.

JORF (2006). Arrêté du 19 octobre 2006 relatif à l'emploi d'auxiliaires technologiques dans la fabrication de certaines denrées alimentaires NOR: ECOC0600115A [Article Annexe I C, enzymes]. Journal Officiel de la République Française, (Oct.). http://www.legifrance.gouv.fr/affichTexte.do?cidTexte=LEGITEXT000020667468.

Kizis D, Natskoulis P, Nychas GJ, Panagou EZ (2014). Biodiversity and ITS-RFLP characterisation of *Aspergillus* section *Nigri* isolates in grapes from four traditional grape-producing areas in Greece. PLoS ONE, 9, e93923. doi:10.1371.

MHLW (1997). Good Laboratory Practice [GLP] Standard Ordinance for Non-Clinical Laboratory Studies on Safety of Drugs. MHW Ordinance no 21, March 26, 1997. Ministry of Health, Labor and Welfare, Japan (MHLW), Tokyo, Japan.

MHLW (2008). Ordinance on the GLP Standard for Conduct of Nonclinical Safety Studies of Drugs. MHW Ordinance no 114, June 13, 2008, amending MHW Ordinance no 21. Tokyo, Japan: Ministry of Health, Labor and Welfare, Japan (MHLW) and Translated by Japan Society of Quality Assurance (JSQA), Tokyo, Japan. http://www.jsqa.com/en/GLP_Ordinance114(Jan17,2011).pdf.

MHLW (2014a). List of existing food additives [Complied and published by the Ministry of Health and Welfare on April 16, 1996]. Tokyo, Japan: Ministry of Health, Labor and Welfare, Japan (MHLW) and Japan Food Chemical Research Foundation (JFCRF). http://www.ffcr.or.jp/zaidan/FFCRHOME.nsf/pages/list-exst.add [Effective from January 30, 2014, Last update: 06/30/2014].

MHLW (2014b). Fructosyl transferase. In: [Japan's Specifications and Standards for Food Additives] (ninth edition). Ministry of Health, Labor and Welfare, Japan (MHLW), Tokyo, Japan, pp. 954-957. http://www.mhlw.go.jp/stf/shingi/0000040629.html.

OECD (1997). Bacterial reverse mutation test. In: OECD Guidelines for the Testing of Chemicals. OECD Guideline no 471 [Updated & Adopted 21st July 1997]. Organisation for Economic Co-Operation and Development (OECD), Paris. http://www.oecd-ilibrary.org/environment/test-no-471-bacterial-reverse-mutation-test_9789264071247-en;jsessionid=1ijgh8o34k0qd.delta.

OECD (1998a). OECD principles of good laboratory practice (as Revised in 1997). OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, no 1 - ENV/MC/CHEM(98)17. Organisation for Economic Co-operation and Development (OECD), Environment Directorate, Chemicals Group and Management Committee, OECD Environmental Health and Safety Publications, Paris. http://www.oecd.org/officialdocuments/displaydocumentpdf/?cote=env/mc/chem(98)17&doclanguage=en.

OECD (1998b). Repeated dose 90-day oral toxicity study in rodents. In: OECD Guidelines for the Testing of Chemicals. OECD Guideline no 408 [Updated & Adopted 21st September 1998]. Organisation for Economic Co-Operation and Development (OECD), Paris. http://www.oecd-ilibrary.org/environment/test-no-408-repeated-dose-90-day-oral-toxicity-study-in-rodents_9789264070707-en;jsessionid=1m6bvwspup322.delta.

OECD (2010). *In Vitro* mammalian cell micronucleus test. In: OECD Guidelines for the Testing of Chemicals. OECD Guideline no 487 [Adopted 22 July 2010]. Organisation for Economic Co-operation and Development (OECD), Paris. http://www.oecd-ilibrary.org/environment/test-no-487-in-vitro-mammalian-cell-micronucleus-test_9789264091016-en.

Samson RA, Houbraken JAMP, Kuijpers AFA, Frank JM, Frisvad JC (2004). [New ochratoxin A or sclerotium producing species in *Aspergillus* section *Nigri*. Studies in Mycology, 50, 45-61.

Spadaro D, Patharajan S, Lorè A, Garibaldi A,Gullino ML (2012). Ochratoxigenic black species of Aspergilli in grape fruits of northern Italy identified by an improved PCR-RFLP procedure. Toxins, 4, 42-54.

Susca A, Proctor RH, Mule G, Stea G, Ritieni A, Logrieco A, Moretti A (2010). Correlation of mycotoxin fumonisin B2 production and presence of the fumonisin biosynthetic gene fum8 in *Aspergillus niger* from grape. Journal of Agricultural and Food Chemistry, 58, 9266-9272.

Téren J, Varga J, Hamari Z, Rinyu E, Kevei F (1996). Immunochemical detection of ochratoxin A in black *Aspergillus* strains. Mycopathologia, 134, 171-176.

U.S. FDA (2000). Agency response letter GRAS Notice No. GRN 000044 [Fructooligosaccharide - GTC Nutrition Company, Golden, CO]. U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Washington, DC. http://www.accessdata.fda.gov/scripts/fdcc/?set=GRASNotices&id=44&sort=GRN_No&order=DESC&startrow=1&type=basic&search=fructo [Nov. 22, 2000].

U.S. FDA (2007). Agency additional correspondence letter: GRAS Notice No. GRN 000044 [Fructooligosaccharide - GTC Nutrition, Golden, CO]. U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), College Park, MD. http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm154400.htm [June 1, 2007].

Varga J, Frisvad JC, Kocsubé S, Brankovics B, Tóth B, Szigeti G, Samson RA (2011). New and revisited species in *Aspergillus* section *Nigri*. Studies in Mycology, 69, 1-17.

WHO/FAO (2009). Foods derived from modern biotechnology (second edition). (Codex Alimentarius). World Health Organization (WHO) / Food and Agriculture Organization of the United Nations (FAO), Rome, Italy. ftp://ftp.fao.org/docrep/fao/011/a1554e/a1554e00.pdf.