ORIGINAL SUBMISSION

GRN 000509

Shin Nihon Chemical Co., Ltd.

19-10, Showa-machi, Anjou, Aichi 446-0063, Japan
Tel: +81-566-75-5555 Fax: +81-566-75-0010

March 26, 2014

RECEIVED
APR 02 2014
OFFICE OF FOOD ADDITIVE SAFETY

Dr. Paulette Gaynor
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Dear Dr. Gaynor:

Re: GRAS Exemption Claim for a Glucose Oxidase Preparation from *Penicillium chrysogenum*

In accordance with proposed 21 CFR §170.36 [Notice of a claim for exemption based on a Generally Recognized as Safe (GRAS) determination] published in the *Federal Register* [62 FR 18938 (17 April 1997)], I am submitting one hard copy and one electronic copy (on CD), as the notifier [Shin Nihon Chemical Co., Ltd., 19-10 Showamachi, Anjou-city Aichi Prefecture Japan 446-0063], a Notice of the determination, on the basis of scientific procedures, that the glucose oxidase preparation from *Penicillium chrysogenum* strain PGO 19-162, produced by Shin Nihon Chemical Co., Ltd., as defined in the enclosed documents, is GRAS under specific conditions of use in food processing, and therefore, is exempt from the premarket approval requirements of the *Federal, Food, Drug and Cosmetic Act.* Information setting forth the basis for the GRAS determination, which includes detailed information on the notified substance and a summary of the basis for the GRAS determination, as well as a consensus opinion of an independent panel of experts in support of the safety of the glucose oxidase preparation under the intended conditions of use, also are enclosed for review by the agency.

An additional hard copy of the GRAS Notice is included for review of the intended use of the glucose oxidase preparation in powdered, dried eggs by the United States Department of Agriculture's (USDA) Food Safety and Inspection Service (FSIS) on the basis that dried eggs meet the definition of egg products in 9CFR §590.5, which are regulated by FSIS.

Should you have any questions or concerns regarding this GRAS Notice, please do not hesitate to contact me at any point during the review process so that we may provide a response in a timely manner.

Sincerely,

(b) (6)

Tetsuya Konishi
Director, Quality Assurance
Shin Nihon Chemical Co., Ltd.

Shin Nihon Chemical Co., Ltd.
19-10, Showa-machi, Anjou, Aichi 446-0063, Japan
Tel: +81-566-75-5555 Fax: +81-566-75-0010

April 1, 2014

Dr. Paulette Gaynor
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Dear Dr. Gaynor:

Re: GRAS Exemption Claim for a Glucose Oxidase Preparation from *Penicillium chrysogenum*

I hereby certify that the enclosed electronic files for the Notification entitled, "GRAS Exemption Claim for a Glucose Oxidase Preparation from Penicillium chrysogenum" were scanned for viruses prior to submission and are thus certified as being virus-free using McAfee VirusScan 8.8.

Sincerely,

(b) (6)

Tetsuya Konishi
Director, Quality Assurance
Shin Nihon Chemical Co., Ltd.

GRN 000509

RECEIVED
APR 02 2014
OFFICE OF
FOOD ADDITIVE SAFETY

GRAS Exemption Claim for a Glucose Oxidase Preparation from *Penicillium chrysogenum*

Submitted to:

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD
U.S.A. 20740-3835

Submitted by:

Shin Nihon Chemical Co., Ltd.
19-10 Showamachi, Anjou city, Aichi prefecture
Japan 446-0063

March 25, 2014

GRAS Exemption Claim for a Glucose Oxidase Preparation from *Penicillium chrysogenum*

Submitted to:

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD
U.S.A. 20740-3835

Submitted by:

Shin Nihon Chemical Co., Ltd.
19-10 Showamachi, Anjou city, Aichi prefecture
Japan 446-0063

March 25, 2014

GRAS Exemption Claim for a Glucose Oxidase Preparation from *Penicillium chrysogenum*

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I GRAS EXEMPTION CLAIM

I.A Claim of Exemption from the Requirement for Premarket Approval Pursuant to Proposed 21 CFR §170.36(c)(1) [62 FR 18938 (17 April 1997)]

Shin Nihon Chemical Co., Ltd. hereby claims that the use of a glucose oxidase preparation from *Penicillium chrysogenum* strain PGO 19-162 in foods, as described in Section I.D below, is exempt from the requirement of premarket approval of the *Federal Food, Drug, and Cosmetic Act* because we have determined that such uses are Generally Recognized as Safe (GRAS).

Signed,

(b) (6)

March 25, 2014

Mr Tetsuya Konishi
Director, Quality Assurance
Shin Nihon Chemical Co., Ltd.
konishi@snc-enzymes.co.jp

Date

I.B Name and Address of Notifier

Shin Nihon Chemical Co., Ltd.
19-10 Showamachi, Anjou-city Aichi Prefecture
Japan 446-0063
Tel: 1-566-75-5555
Fax: 1-566-75-0010

I.C Common Name of the Notified Substance

Glucose oxidase preparation from *Penicillium chrysogenum* strain PGO 19-162

I.D Conditions of Intended Use in Food

I.D.1 Technological Function

The technological function of glucose oxidase is to break down the residual glucose and oxygen in foods and beverages by catalyzing the oxidation of β-D-glucose to D-glucono-1,5-lactone (D-glucono-δ-lactone) in the presence of molecular oxygen, releasing hydrogen peroxide (H_2O_2) in the process. The enzymatic reaction catalyzed by glucose oxidase is described by the following reaction:

$$\beta\text{-D-glucose} + O_2 \xrightarrow{\text{glucose oxidase}} \text{D-glucono-1,5-lactone} + H_2O_2$$

Glucose oxidase serves as a preservative and stabilizer by the mechanism described above, and also is used to strengthen the protein complexes contained in starch-based products. A complete description of the established technological function and current food and beverage applications of glucose oxidase is provided in Section IV.A.1.

I.D.2 Food Applications and Maximum Levels of Use

The glucose oxidase preparation from *P. chrysogenum* strain PGO 19-162 is intended for use as a processing aid with uses that are consistent with glucose oxidases from other sources used in current food and beverage applications (see Section IV.A.1). The proposed food uses and maximum use levels of the glucose oxidase preparation are presented in Table I.D.2-1. The maximum level of use of the enzyme preparation expressed on a total organic solids (TOS) basis is 30 mg/kg in foods and 3 mg/kg in beverages. The enzyme preparation will be used only at the minimum level necessary to achieve the intended technical effect. Of note, powdered dried eggs, powdered dried egg whites, and powdered dried egg yolks meet the definition of egg products in Title 9 of the *Code of Federal Regulations* (CFR), Section 590.5 (FSIS/USDA, 2013), which are regulated by the United States Department of Agriculture's (USDA) Food Safety and Inspection Service (FSIS). The use of the glucose oxidase preparation from *P. chrysogenum* strain PGO 19-162 in powdered dried eggs, powdered dried egg whites, and powdered dried egg yolks would therefore fall under the regulatory authority of FSIS. Furthermore, standards of identity exist for dried eggs, dried egg whites, and dried egg yolks under 21CFR §160.105, 160.145, 160.185, respectively (U.S. FDA, 2013). Under these standards, glucose oxidase-catalase preparations are permitted for use in the manufacture of dried whole eggs, dried egg whites, and dried egg yolks for the purpose of reducing the glucose content of the liquid prior to drying so long as the enzyme preparation is GRAS within the meaning of Section 201(s) of the *Federal Food, Drug, and Cosmetic Act* (U.S. FDA, 2012). The use of the glucose oxidase preparation from *P. chrysogenum* strain PGO 19-162 is therefore considered acceptable in the manufacture of these products on the basis that the enzyme preparation is GRAS as described herein.

Table I.D.2-1 Proposed Food Uses for Glucose Oxidase Preparation from *Penicillium chrysogenum* Strain PGO 19-162 Expressed as Total Organic Solids (TOS) in the United States

Food Category	Proposed Food Use	Maximum TOS Content (mg/kg food)
Alcoholic Beverages	Cocktail drinks	3
	Distilled Liquors	3
	Wine	3
Baked Goods and Baking Mixes	Bagels	30

Table I.D.2-1 Proposed Food Uses for Glucose Oxidase Preparation from *Penicillium chrysogenum* Strain PGO 19-162 Expressed as Total Organic Solids (TOS) in the United States

Food Category	Proposed Food Use	Maximum TOS Content (mg/kg food)
	Biscuits	30
	Breads and Rolls	30
	Cakes	30
	Cookies	30
	Cornbread, Corn Muffins, and Tortillas	30
	Crackers	30
	Croissants and Pastries	30
	Doughnuts	30
	French Toast, Pancakes and Waffles	30
	Muffins	30
	Pies	30
Beverages and Beverage Bases	Carbonated Beverages	3
	Energy, Sports, and Isotonic Drinks	3
	Enhanced and Fortified Water	3
	Meal Replacements, Non-Milk Based	3
	Non-Carbonated Beverages	3
Coffee and Tea	Coffee and Coffee-Based Drinks	3
	Coffee Substitutes	3
	Tea and Tea-Based Drinks	3
Condiments and Relishes	Ketchup	30
	Mustard	30
	Other Condiments and Relishes	30
Egg Products	Powdered Eggs	30
Fats and Oils	Mayonnaise	30
Gelatins, Puddings, and Fillings	Fruit Fillings	30
Grain Products and Pastas	Energy, Meal Replacement, and Fortified Bars	30
	Frozen Multi-Course Meals Containing Pasta	30
	Granola and Cereal Bars	30
	Macaroni and Noodle Products	30
	Rice	30
Processed Fruits and Fruit Juices	Canned Fruits	30
	Fruit-Based Desserts	30
	Fruit Drinks and Ades	3
	Fruit Juices	3
	Fruit Smoothies	3
	Nectars	3
Processed Vegetables and Vegetable Juices	Vegetable Juices	3
	Vegetable Purees	3
Snack Foods	Savory Snacks	30

I.E Basis for the GRAS Determination

Pursuant to Title 21, Section 170.30 of the *Code of Federal Regulations* (CFR) (U.S. FDA, 2013), glucose oxidase preparation from *P. chrysogenum* strain PGO 19-162 has been determined to be GRAS based on scientific procedures.

The data and information that serve as the basis for this GRAS Notification will be sent to the U.S. Food and Drug Administration (FDA) upon request, or will be available for review and copying at reasonable times at the offices of:

Shin Nihon Chemical Co., Ltd.
19-10 Showamachi, Anjou city, Aichi prefecture
Japan 446-0063

Should the FDA have any questions or additional information requests regarding this notification, Shin Nihon Chemical Co., Ltd. will supply these data and information.

II DETAILED INFORMATION ABOUT THE IDENTITY OF THE SUBSTANCE

II.A Identity

II.A.1 Identity of the Enzyme

Glucose oxidase is identified by the following names and systematic numbers:

Accepted Name:	Glucose oxidase
Systematic Name:	β-D-glucose:oxygen-1-oxidoreductase
Synonyms:	Glucose oxyhydrase; glucose aerodehydrogenase; β-D-glucose oxidase; D-glucose oxidase; D-glucose-1-oxidase; β-D-glucose:quinone oxidoreductase; glucose oxyhydrase
IUBMB Number: **[Enzyme Commission (EC) Number]**	EC 1.1.3.4
Chemical Abstracts Service (CAS) Number:	9001-37-0

II.A.2 Amino Acid Sequence

The glucose oxidase enzyme from *P. chrysogenum* PGO 19-162 has a calculated molecular weight of 64,490 Da. The full length amino acid (aa) sequence of the glucose oxidase enzyme from *P. chrysogenum* PGO 19-162 is as follows:

YSPAEQIDVQSHLLSDPTKVEGETYDYVIAGGGLTGLTVAAKLSENPKIKVLVIEKGFYESNDGPIIED
PNAYGEIFGTSVDQNYLTVPLINNRTGEIKSGLGLGGSTLINGDSWTRPDKVQIDSWEKVFGMEGW
NWDNVFQYMQKAERSRPPTAAQIEAGHFYDPACHGTDGTVHAGPRDNGKPWSPLMRALMNTVSA
FGVPVQKDFHCGHPRGVSMIPNNLHENQIRADAAREWLLPNYQRDNLQILTGQKVGKVLFNQTAS
GPKAVGVNFGTNKAVNFNVYAKQEVLLAAGSAISPLILEYSGIGIKSVLDKAGVKQLLELPVGLNMQD
QTTTTVRSRANNAPGQGQAAYFANFTEVLGDHAAQGIKLLDTKLDQWAEETVARGGFHNVTALKIQ
YENYRNWLLDEDVAFAELFFDTEGKINFDIWNLIPFTRGSVHILSSDPYLWQYANDPKFFMNELDLL
GQAAATKLGRELSSAGEMKKYYAGETIPGDNLPQDATVEQWEDYVMMNFRPNWHAVSTCSMMSR
ELGGVVDATAKVYGTQGLRVIDGSIPPTQVSSHVMTVFYGMALRIAESVLEDYAKKA

The 604 aa sequence for the glucose oxidase enzyme derived from *P. chrysogenum* PGO 19-162 is identical to a 604 aa protein product associated with the gene locus tag Pc20g09560 from the *P. chrysogenum* reference genome (strain Wisconsin 54-1255) (accession number CAP86285.1). This 604 aa sequence has a 99% sequence similarity to the sequence for glucose oxidase from *P. chrysogenum* (accession number AFA42947; 604 aa sequence) recorded in the National Center of Biotechnology Information (NCBI) protein database [as determined through a sequence alignment search against the non-redundant protein databases available through the NCBI using the Basic Local Alignment Search Tool (BLAST) program blastp].

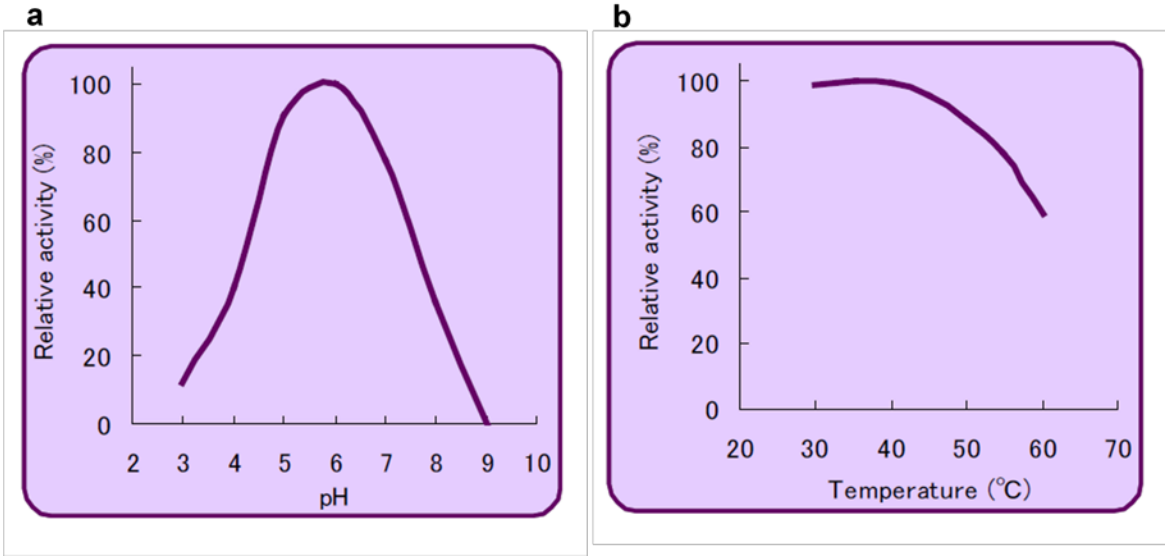
II.A.3 Enzyme Properties

Glucose oxidase catalyzes the oxidation of β-D-glucose to D-glucono-1,5-lactone (D-glucono-δ-lactone) in the presence of molecular oxygen, which serves as an electron acceptor. D-glucono-1,5-lactone is subsequently hydrolyzed to gluconic acid by non-enzymatic means. Hydrogen peroxide (H_2O_2) also is produced as a by-product of the reaction. The enzymatic reaction catalyzed by glucose oxidase is described by the following reaction:

$$\beta\text{-D-glucose} + O_2 \xrightarrow{\text{glucose oxidase}} \text{D-glucono-1,5-lactone} + H_2O_2$$

The glucose oxidase enzyme derived from *P. chrysogenum* PGO 19-162 has an activity that is specific to glucose and does not act on other monosaccharide or disaccharide substrates (*i.e.*, galactose, mannose, maltose, fructose, sucrose, lactose). As illustrated in Figure II.A.3-1, the enzyme functions optimally at a pH of 6 and at a temperature of 37°C (0.2 U/mL enzyme solution). The enzyme becomes thermally unstable at temperatures in excess of 50°C (see Section II.G.2), and may be inactivated by heating to a temperature of 80°C and holding for 15 minutes.

Figure II.A.3-1 Activity of glucose oxidase from *Penicillium chrysogenum* PGO 19-162 as a function of a) pH and b) temperature



II.B Production Organism

The production strain from which the glucose oxidase enzyme is produced is a non-genetically modified strain of *P. chrysogenum*, designated as strain PGO 19-162. The strain was originally isolated from a soil sample following growth on potato dextrose agar plates in the presence of chloramphenicol. Fungal colonies were selected and analyzed for glucose oxidase activity. Strain PGO 19-162 was selected as the production strain based on its capacity to produce high levels of glucose oxidase activity.

The strain was taxinomically identified as belonging to the species *P. chrysogenum* by the CABI Microbial Identification Service using morphological methods consisting of macroscopic and microscopic analysis on diagnostic media and use of taxonomic keys (personal communication to Shin Nihon). The strain has been deposited in the CABI Genetic Resource Collection as a strain of *P. chrysogenum*. Shin Nihon further outsourced the taxonomic identification of the strain to TechnoSuruga Laboratory (Shizuoka, Japan), who further identified the strain as belonging to the species *P. chrysogenum*[1] by phylogenetic means based on β-tubulin gene sequence similarities. Briefly, genomic deoxyribonucleic acid (DNA) from strain PGO 19-162 was extracted and the gene encoding for β-tubulin was amplified by polymerase chain reaction

[1] Several strains of *Penicillium chrysogenum*, including strain Wisconsin 54-1255 (*P. chrysogenum* reference genome), were recently re-identified as belonging to the species *Penicillium rubens* by Houbraken and colleagues (Houbraken *et al*., 2011, 2012). As a result of this re-identification, TechnoSuruga Laboratory identified Shin Nihon's strain PGO 19-162 as belonging to the group of *P. chrysogenum* strains now classified under *P. rubens*. For the purposes of this GRAS notice, strain PGO 19-162 is treated as a strain of *P. chrysogenum* on the basis that the data on the group of strains to which strain PGO 19-162 belongs have been historically documented under the species *P. chrysogenum*.

(PCR) and sequenced. The sequence was then subjected to an alignment search against nucleotide sequences available through the International Nucleotide Sequence Database Collaboration [comprising the DNA DataBank of Japan (DDBJ), the European Molecular Biology Laboratory (EMBL), and GenBank at NCBI] using the BLAST program. The alignment search revealed a 100% sequence similarity to the β-tubulin gene of several strains of *P. chrysogenum* (including strain Wisconsin 54-1255). Based on this high sequence similarity, and on subsequent phylogenetic analysis, strain PGO 19-162 was identified as belonging to the species *P. chrysogenum*.

II.C Method of Manufacture

II.C.1 Raw Materials and Processing Aids

The raw materials and processing aids used in the manufacture of the glucose oxidase preparation are listed in Table II.C.1-1. All raw materials and processing aids are food-grade and comply with purity criteria and limits established in the Food Chemicals Codex (FCC), where applicable, or equivalent. All filtration aids are those commonly used by the food industry in the purification of food ingredients and are permitted for use in the U.S. for such purposes. Maltodextrin is present in the final enzyme preparations as a diluent and is affirmed as GRAS for use in food with no limitation other than current good manufacturing practices (cGMP) (21CFR §184.1444) (U.S. FDA, 2013).

Table II.C.1-1 Raw Materials and Processing Aids Used in the Manufacture of Glucose Oxidase Preparation from *Penicillium chrysogenum* Strain PGO 19-162

Material	Function
Culture Medium Materials	
Sucrose	Nutrient in culture medium
Soybean meal	Nutrient in culture medium
Potassium dihydrogen phosphate[a]	Mineral source in culture medium
Processing Aid	
Ethanol	To precipitate glucose oxidase
Filtration Aids	
Diatomaceous earth[a]	Filtration aid
Polyethersulfone membrane	Ultrafiltration
Polypropylene filter	Filtration
Ceramic filter – diatomaceous earth based	Filter
Diluent	
Maltodextrin	To adjust enzyme activity level

[a] Complies with the corresponding monograph in Japan's Specifications and Standards for Food Additives (7th Edition) (MHLW, 2000).

II.C.2 Manufacturing Process

The glucose oxidase preparation is manufactured in compliance with cGMP and the principles of Hazard Analysis and Critical Control Points (HACCP). A schematic overview of the manufacturing process is provided in Figure II.C.2-1. The enzyme preparation is produced using food-grade materials and using quality-controlled fermentation and purification/recovery processes.

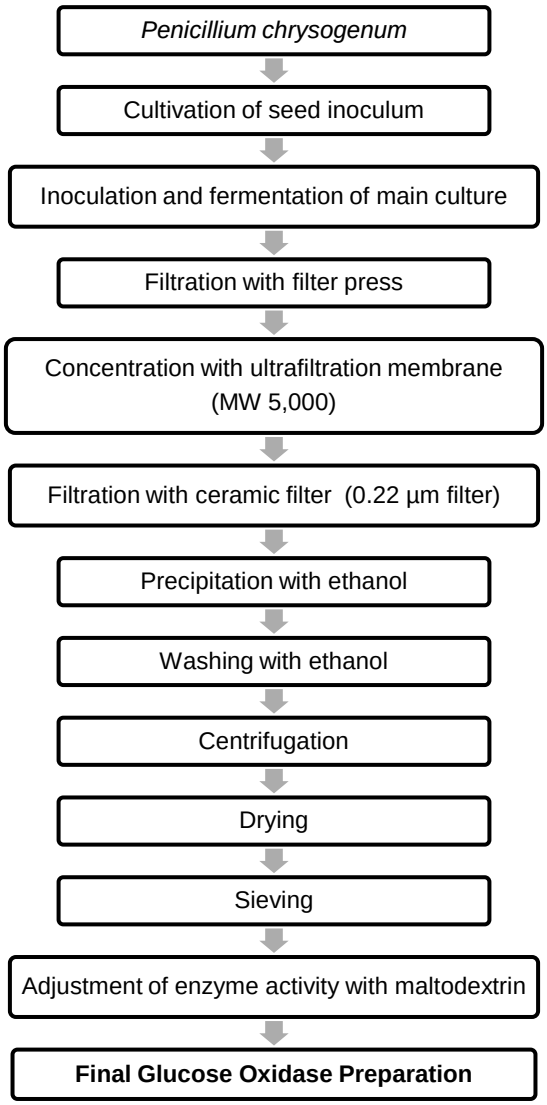
Both the seed inoculum and main culture are grown in sucrose- and soybean meal-based media. A *P. chrysogenum* PGO 19-162 seed inoculum is first cultivated and is then used to inoculate the main liquid culture medium, which is cultivated under submerged fermentation. The glucose oxidase enzyme is secreted from the production strain during fermentation into the culture medium.

Fermentation is followed by a purification process consisting of a series of filtration steps. The first step involves the addition of diatomaceous earth to the main culture and filtering the mixture through a polypropylene filter press to remove the production strain. The filtrate containing the enzyme is concentrated by ultrafiltration using a polyethersulfone membrane (pore size of 5,000 MW), which removes any proteins, saccharides, lipids, salts, and other compounds less than 5,000 MW. The concentrated solution is then passed through a ceramic filter (pore size of 0.22 µm) to remove any potential contaminating microorganisms and residual amounts of the production strain.

Following purification, ethanol is added to the filtered solution to precipitate the enzyme, and the resultant slurry is washed with ethanol. The slurry is then centrifuged to isolate the precipitated solid enzyme material, and the supernatant, which consists of more than 92% ethanol, is removed and discarded. The solid precipitate is then dried by vacuum. Alternatively, the filtered enzyme solution is dried by a spray drying process using a fluid-bed dryer.

The dried enzyme is sifted through an 80-mesh (180 µm) sifter to obtain a powdered material. The final concentrated, unstandardized, powdered enzyme product possesses a glucose oxidase activity of 60,000 U/g. The glucose oxidase activity of the powdered product is adjusted with the addition of maltodextrin to produce the final formulated enzyme preparations. The final enzyme preparations are powdered products that are white to light tan in color. Prior to release, the enzyme preparations are tested to ensure compliance with the product specifications.

Figure II.C.2-1 Schematic Overview of the Manufacturing Process of Glucose Oxidase Preparation from *Penicillium chrysogenum* Strain PGO 19-162



II.C.3 Quality Control

Quality control steps are included throughout the fermentation and purification processes in order to ensure adherence with the established production process and to produce a high quality and consistent product. Chemical and microbiological analyses are performed on the final enzyme preparation to ensure absence of heavy metal and microbiological contaminants, as outlined in Section II.E below. Items that are monitored and controlled during the production process and the corresponding acceptance criterion are listed in Table II.C.3-1 and include measures to ensure that residual amounts of the *P. chrysogenum* production strain are not

transferred to the final enzyme preparation. Methods of analysis are the same as those for the final enzyme preparations, which are listed in Section II.E.

Table II.C.3-1 Quality Control Steps in the Production of Glucose Oxidase Preparation from *Penicillium chrysogenum* Strain PGO 19-162

Quality Control Step	Items Measured or Controlled[a]	Criterion
Production strain	n/a	Stored in CABI culture collection Stored at -80°C in production facility
Seed culture	Production strain	More than 10^6
	Total aerobic plate count	Not detected
Inoculated main culture	Glucose oxidase activity	>300 U/g
Concentration with ultrafiltration membrane	Glucose oxidase activity	>3000 U/g
Filtration with ceramic filter	Production strain	Not detected
	Total aerobic plate count	<50,000 CFU/g
	Glucose oxidase activity	>,3000 U/g
Drying	Production strain	Not detected
	Total aerobic plate count	<50,000 CFU/g
	Glucose oxidase activity	>60,000 U/g
Sieving	Production strain	Not detected
	Total aerobic plate count	<50,000 CFU/g
Final glucose oxidase preparation	n/a	Meets product specifications[b]

CFU = colony forming units; n/a = not applicable
[a] Methods of analysis are listed in Section II.E.
[b] Product specifications and methods of analysis are presented in Section II.E.

II.D Composition of the Enzyme Preparation

The final glucose oxidase preparation is formulated with a specific activity of either 2,000 or 15,000 U glucose oxidase activity/g. As mentioned, glucose oxidase activity is standardized to the desired level with the use of food-grade maltodextrin. Maltodextrin is affirmed as GRAS in the U.S. for use in foods, with no limitation other than cGMP (21CFR §184.1444) (U.S. FDA, 2013). No other diluents, stabilizers, or preservatives are added to formulate the enzyme preparations. The composition of the final two enzyme preparations is presented in Table II.D-1.

Table II.D-1 Composition of Glucose Oxidase Preparations from *Penicillium chrysogenum* Strain PGO 19-162

Parameter	Content
Preparation with a glucose oxidase activity of 2,000 U/g	
Glucose oxidase specific activity	Not less than 2,000 U/g
Total organic solids	2.0 to 3.0%
Water	0.06
Ash	0.44
Maltodextrin	97 to 97.5%
Preparation with a glucose oxidase activity of 15,000 U/g	
Glucose oxidase specific activity	Not less than 15,000 U/g
Total organic solids	15 to 25%
Water	0.5
Ash	3.5
Maltodextrin	77 to 82 %

II.E Specifications for Food-Grade Material

Appropriate food-grade product specifications have been established for the final glucose oxidase preparations and are presented in Table II.E-1 below. The specifications for both enzyme preparations are identical apart from glucose oxidase activity and TOS content. The specifications comply with the current purity and microbial limits established for enzyme preparations in the FCC (2012) and by the Joint FAO/WHO Committee on Food Additives (JECFA) (2006a). The specifications also include a specification for mold, as well as for the mycotoxin roquefortine C, which may be produced by the production strain (see Sections II.F.2 and IV.B.1.2 for a discussion on mycotoxin production). Each batch of the glucose oxidase preparation is certified to contain a minimum glucose activity of either 2,000 or 15,000 U/g, depending on the formulation. All methods of analysis are nationally or internationally recognized or have been validated by Shin Nihon.

Table II.E-1 Specifications for Glucose Oxidase Preparations from *Penicillium chrysogenum* Strain PGO 19-162

Specification Parameter	Specification Requirements	Method of Analysis
Assay (Activity)	2,000 U glucose oxidase activity/g preparation: NLT 2,000 U/g	Glucose oxidase assay (in-house method)
	15,000 U glucose oxidase activity/g preparation: NLT 15,000 U/g	
Enzyme solids (total organic solids)	2,000 U glucose oxidase activity/g preparation: 2.0 to 3.0%	Calculation[a] (JECFA, 2006a)
	15,000 U glucose oxidase activity/g preparation: 15 to 25%	
Roquefortine C	Not detected[b]	Modified method of O'Brien *et al*. (2006)
Arsenic	NMT 3 mg/kg	DDTC-Ag colorimetric method (FCC, 2012)
Lead	NMT 1 mg/kg	AAS (FCC, 2012)
Total aerobic plate count	NMT 50,000 CFU/g	BAM – Chapter 3: Conventional plate count method (U.S. FDA, 2001)
Coliforms	NMT 30 CFU/g	BAM – Chapter 4: Conventional method for coliforms (U.S. FDA, 2001)
Escherichia coli	Negative in 25 g	BAM – Chapter 4: Conventional method for *E. coli* (U.S. FDA, 2001)
Salmonella species	Negative in 25 g	AOAC Method 989.13 (AOAC, 2000)
Mold	NMT 100 CFU/g	BAM – Chapter 18: Dilution plating technique (U.S. FDA, 2001)
Antibacterial activity	Negative	Antibacterial activity (JECFA, 2006b)

AAS = atomic absorption spectrophotometry; AOAC = Association of Analytical Communities; BAM = Bacteriological Analytical Manual; CFU = colony forming units; DDTC-Ag = silver diethyldithiocarbamate; JECFA = Joint FAO/WHO Expert Committee on Food Additives; NLT = not less than; NMT = not more than; U.S. FDA = United States Food and Drug Administration

[a] Total organic solids = 100% - (A+W+D), where A = % ash, W = % water, and D = % diluents and/or other formulation ingredients.

[b] Limit of quantitation = 0.25 ppm

Analytical results for 3 non-consecutive sample lots of each enzyme preparation manufactured by Shin Nihon are presented in Table II.E-2 and Table II.E-3. The results verify that the manufacturing process produces a consistent product that meets the product specifications. To note, while analytical results for roquefortine C are currently only available for the concentrated non-standardized enzyme preparation (with a glucose oxidase activity of 60,000 U/g) (see Section II.F.2), batches of the final enzyme preparations marketed in the U.S. will be subjected to roquefortine C analysis to ensure conformity to the product specifications.

Table II.E-2 Summary of the Product Analysis for 3 Lots of the Glucose Oxidase Preparation from *Penicillium chrysogenum* Strain PGO 19-162 with a Glucose Oxidase Activity of 2,000 U/g

Specification Parameter[a]	Specification	Manufacturing Lot		
		060722-02[a]	111102-02[b]	120229-01[c]
Assay (Activity)	NLT 2,000 U/g	2,000	2,000	2,000
Total organic solids	2.0 to 3.0%	2.7	2.5	2.7
Arsenic	NMT 3 mg/kg	Not detected[d]	Not detected[d]	Not detected[d]
Lead	NMT 1 mg/g	Not detected[e]	Not detected[e]	Not detected[e]
Total viable plate count	NMT 50,000 CFU/g	<10	<10	<10
Coliforms	NMT 30 CFU/g	<30	<30	<30
Escherichia coli	Negative in 25 g	Negative	Negative	Negative
Salmonella species	Negative in 25 g	Negative	Negative	Negative
Mold	NMT 100 CFU/g	<10	<10	<10
Antibacterial activity	Negative	Negative	Negative	Negative

CFU = colony forming units; NLT = not less than; NMT = not more than
[a] Date of manufacture: 22/07/06
[b] Date of manufacture: 02/11/11
[c] Date of manufacture: 29/02/12
[d] Arsenic value was below the limit of detection of 1.0 mg/kg.
[e] Lead value was below the limit of detection of 0.1 mg/kg.

Table II.E-3 Summary of the Product Analysis for 3 Lots of the Glucose Oxidase Preparation from *Penicillium chrysogenum* Strain PGO 19-162 with a Glucose Oxidase Activity of 15,000 U/g

Specification Parameter[a]	Specification	Manufacturing Lot		
		101001-02[a]	110416-02[b]	120128-02[c]
Assay (Activity)	NLT 15,000 U/g	15,000	15,000	15,000
Total organic solids	15 to 25%	18.1	20.5	19.8
Arsenic	NMT 3 mg/kg	Not detected[d]	Not detected[d]	Not detected[d]
Lead	NMT 1 mg/kg	Not detected[e]	Not detected[e]	Not detected[e]
Total viable plate count	NMT 50,000 CFU/g	<10	<10	<10
Coliforms	NMT 30 CFU/g	<30	<30	<30
Escherichia coli	Negative in 25 g	Negative	Negative	Negative
Salmonella species.	Negative in 25 g	Negative	Negative	Negative
Mold	NMT 100 CFU/g	<10	<10	<10
Antibacterial activity	Negative	Negative	Negative	Negative

CFU = colony forming units; NLT = not less than; NMT = not more than
[a] Date of manufacture: 10/10/10
[b] Date of manufacture: 16/04/11
[c] Date of manufacture: 28/01/12
[d] Arsenic value was below the limit of detection of 1.0 mg/kg.
[e] Lead value was below the limit of detection of 0.1 mg/kg.

II.F Additional Chemical Characterization

II.F.1 Solvent Residues

As ethanol is used in the manufacture of the glucose oxidase preparation, testing for ethanol residues was performed to ensure the absence of ethanol residues in the final product. Testing was conducted on 3 non-consecutive lots of the concentrated non-standardized enzyme preparation, with a glucose oxidase activity of 60,000 U/g (Lot Nos. 110713-01, 121031-01, and 120222-01), by gas chromatography. Ethanol levels were below the limit of detection (*i.e.*, 0.1 g/100 g) in all 3 lots, demonstrating a lack of detectable residual ethanol levels prior to formulation of the final enzyme preparations. The results therefore indicate that no detectable residual ethanol would be present in the final diluted standardized enzyme preparations.

II.F.2 Mycotoxins

P. chrysogenum is known to produce the mycotoxin roquefortine C (Frisvad *et al*., 2004). An analysis of the secondary metabolite profile of *P. chrysogenum* PGO 19-162 confirmed that the production strain also produces roquefortine C (see Section IV.B.1.2 for a detailed discussion on mycotoxin production). Therefore, the glucose oxidase preparation was analyzed for roquefortine C to ensure absence of the mycotoxin in the final product. Testing was conducted on 3 non-consecutive lots of the concentrated non-standardized enzyme preparation, with a glucose oxidase activity of 60,000 U/g, by a modified method of that described by O'Brien *et al*. (2006). As shown in Table II.F2-1, the levels of roquefortine C were below the limit of detection in all 3 lots tested, demonstrating a lack of detectable roquefortine C levels prior to formulation of the final enzyme preparations. The analytical results therefore demonstrate that although *P. chrysogenum* PGO 19-162 may produce the mycotoxin roquefortine C, the purification processes employed in the manufacture of the glucose oxidase preparation ensures that the mycotoxin would not be present at detectable levels in the final enzyme preparations. As mentioned, while analytical results for roquefortine C are currently only available for the concentrated non-standardized enzyme preparation, batches of the final enzyme preparations marketed in the U.S. will be subjected to roquefortine C analysis to ensure conformity to the product specifications. No other mycotoxins, including the major food-contaminating mycotoxins of concern to food safety, are produced by the production strain *P. chrysogenum* PGO 19-162.

Table II.F.2-1 Summary of Mycotoxin Analysis for 3 Lots of Concentrated Non-standardized Glucose Oxidase Preparation from *Penicillium chrysogenum* Strain PGO 19-162 with a Glucose Oxidase Activity of 60,000 U/g

Parameter	Limit of Detection	Manufacturing Lot		
		110504R1-01	100119R1-01	070606-03
Roquefortine C	0.25 ppm	not detected[a]	not detected[b]	not detected[c]

Ochratoxin A	0.5 ppb	not detected	not detected	not detected
Aflatoxin (B_1, B_2, G_1, G_2)	0.5 ppb	not detected	not detected	not detected
Zearalenone	50 ppb	not detected	not detected	not detected
Sterigmatocystin	20 ppb	not detected	not detected	not detected
T-2 toxin	50 ppb	not detected[d]	not detected	not detected

[a] Lot No. 130418Z1-01
[b] Lot No. 130214R1-01
[c] Lot No. 121107R1-01
[d] New limit of detection = 0.1 ppm

The enzyme preparation also was analyzed for the major food-contaminating mycotoxins ochratoxin A, aflatoxin (B_1, B_2, G_1, G_2), zearalenone, sterigmatocystin, and T-2 toxin to confirm that these major food-contaminating mycotoxins of concern to food safety are not of concern with respect to the glucose oxidase preparation from *P. chrysogenum* PGO 19-162. In fact, such mycotoxins are not known to be produced by *P. chrysogenum* and the secondary metabolite analysis of strain PGO 19-162 confirmed that the production strain does not produce these mycotoxins. For instance, while ochratoxin A is produced by some species of the *Penicillium* genus (Moss, 2002; Murphy *et al.*, 2006), only *P. nordicum* and *P. verrucosum* are known to produce ochratoxins (Frisvad *et al.*, 2004). Moreover, aflatoxins are produced only by *Aspergillus*, mainly strains belonging to the species *Aspergillus flavus* and *A. parasiticus* (Bennett and Klich, 2003), zearalenone and T-2 toxin are produced only by *Fusarium* species (Bennett and Klich, 2003), and sterigmatocstin is not produced by *Penicillium* (Frisvad *et al.*, 2004; Rank *et al.,* 2011). To confirm absence of the major food-contaminating mycotoxins in the glucose oxidase preparation, mycotoxin testing was conducted on 3 non-consecutive lots of the concentrated non-standardized enzyme preparation, and the results presented in Table II.F.2-1 above. The analytical data confirm that there are no detectable levels of the mycotoxins tested in the concentrated enzyme preparation, and that therefore, the final standardized enzyme preparations would not contain these mycotoxins.

II.G Stability

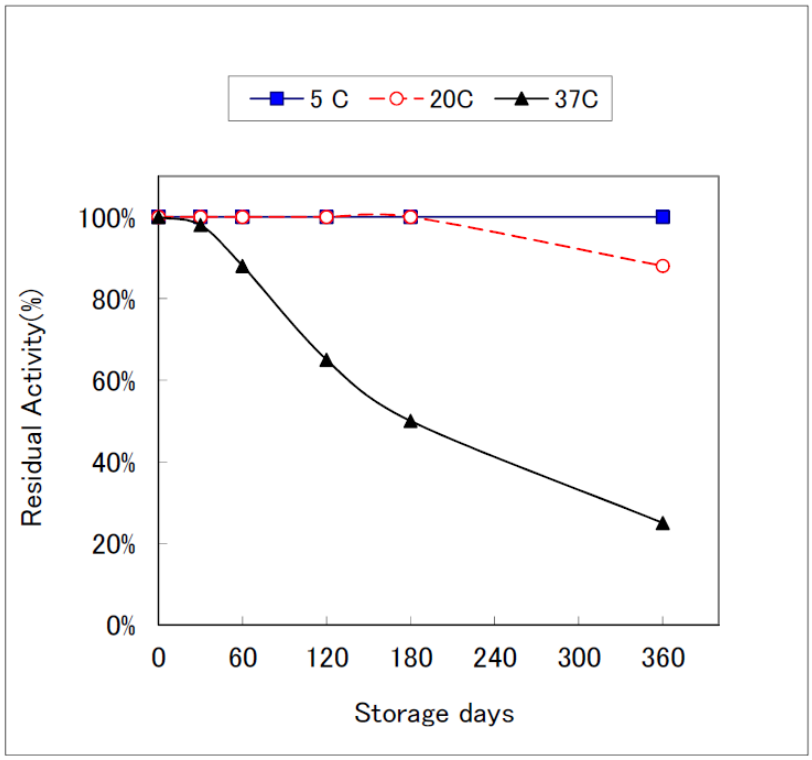
II.G.1 Bulk Stability

The glucose oxidase preparations are stored in fiber drums lined with polyethylene bags and sealed with a ring seal. The product is stored in sealed containers under cool, dry conditions. Under these conditions, glucose oxidase activity is stable for at least 6 months. The product also may be stored under refrigerated conditions (*i.e.*, 5°C), which may extend the storage life of the product. Analytical results supporting the stability of the bulk material under the intended storage conditions are presented in Table II.G.1-1 and Figure II.G.1-1. A sample lot of the enzyme preparation with a glucose activity of 15,000 U/g was tested in the bulk stability experiment.

Table II.G.1-1 Bulk Stability of Glucose Oxidase from *Penicillium chrysogenum* Strain PGO 19-162 with a Glucose Oxidase Activity of 15,000 U/g

Temperature (°C)	Residual Activity (%)					
	Day 0	Day 30	Day 60	Day 120	Day 180	Day 360
5	100	100	100	100	100	100
20	100	100	100	100	100	88
37	100	98	88	65	50	25

Figure II.G.1-1 Bulk Stability of Sumizyme PGO 15000 glucose oxidase activity

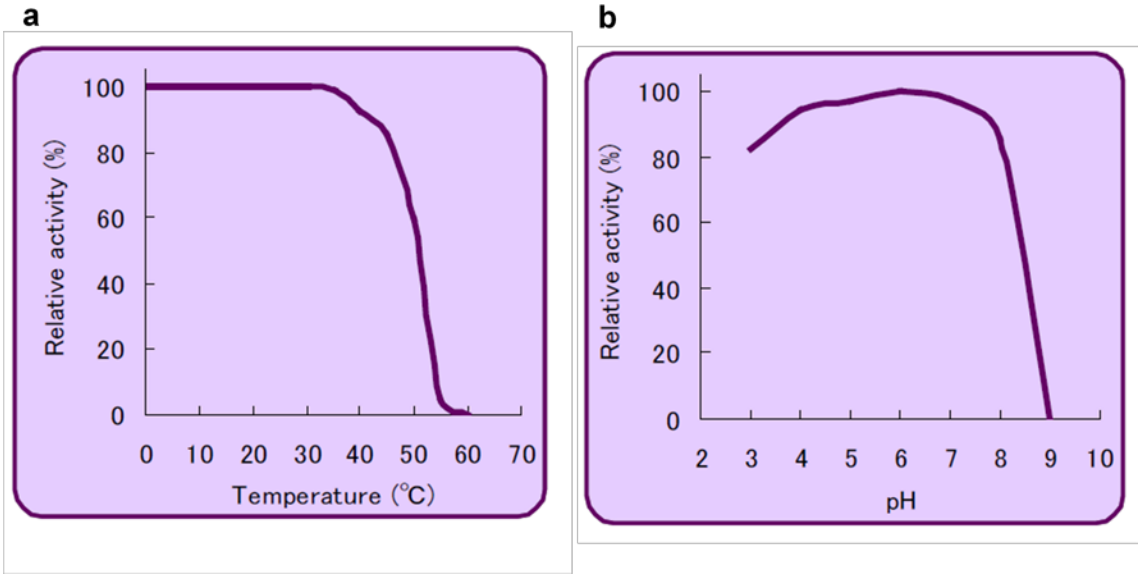


II.G.2 Stability under Varying Conditions

To assess the stability of the glucose oxidase enzyme from *P. chrysogenum* PGO 19-162 under varying temperature conditions, 1 g of an enzyme preparation was dissolved in 100 mL of buffer, resulting in a solution with a pH of 5.5 and with an enzyme activity of 20 U/mL (approximately equivalent to 0.03% TOS/mL). The solution was stored for 5 minutes at the following temperatures: 0, 10, 20, 30, 40, 50, 60, and 70°C) and stability measured by measuring glucose oxidase activity. As shown in Figure II.G.2-1a, glucose oxidase activity was stable at temperatures of 0 to 40°C. Glucose oxidase activity rapidly decreased at higher temperatures and was negligible at a temperature of 60°C.

The stability of the enzyme also was assessed under varying pH conditions. Enzyme solutions were prepared as above at pHs ranging from 3 to 9 and incubated at 30°C for 1 hour. As shown in Figure II.G.2b, glucose oxidase activity was stable at pHs of 4 to 8. Glucose oxidase activity was reduced under acidic conditions (pH 3) and also rapidly decreased to negligible levels at pH 9.

Figure II.G.2-1 Stability of glucose oxidase from *Penicillium chrysogenum* strain PGO 19-162 under varying a) temperature and b) pH conditions.



III SELF-LIMITING LEVELS OF USE

No self-limiting levels of use are associated with the glucose oxidase enzyme preparation from *P. chrysogenum* PGO 19-162.

IV BASIS FOR GRAS DETERMINATION

The data and information summarized below demonstrate that the glucose oxidase enzyme preparation from *P. chrysogenum* PGO 19-162, under the proposed conditions of use described in Table I.D.2-1, is GRAS based on scientific procedures. The estimated intake of the enzyme preparation under the conditions of proposed use was assessed on a TOS basis and the data are presented in Section IV.A. Safety of the proposed use of the glucose oxidase preparation is based on generally available information demonstrating safety of the production strain and use of appropriate purifications steps during the manufacture of the enzyme preparation to ensure absence of mycotoxins in the final product as detailed in Section IV.B.1. The general recognition of safety of the enzyme preparation is further supported by the results of published toxicological studies on the enzyme preparation, which are summarized in Section IV.B.2.

Moreover, this data and information were reviewed by a Panel of Experts, qualified by scientific training and experience to evaluate the safety of the glucose oxidase enzyme preparation from *P. chrysogenum* PGO 19-162, who concluded that the enzyme preparation was GRAS under the aforementioned conditions of intended use in food based on scientific procedures.

IV.A Probable Consumption

IV.A.1 Technological Function and Current Applications in Food

The glucose oxidase preparation from *P. chrysogenum* strain PGO 19-162 is intended for use as a processing aid with uses consistent with the current applications of glucose oxidase in foods and beverages. As mentioned, the technological function of glucose oxidase is to break down the residual glucose and oxygen in foods and beverages by catalyzing the oxidation of β-D-glucose to D-glucono-1,5-lactone in the presence of molecular oxygen, releasing H_2O_2 in the process.

Current applications of glucose oxidase in the food and beverage industry include use as a preservative and stabilizer. The enzyme, in conjunction with catalase, is often applied to remove residual glucose and oxygen in foods and beverages to prolong shelf-life (as reviewed by Crueger and Crueger, 1990; Wong *et al*., 2008 and Bankar *et al*., 2009). The removal of glucose prevents the non-enzymatic browning of foods induced by the Maillard reaction, which may occur during food processing (*e.g.*, pasteurization of powdered eggs and fruit processing) and preparation (*i.e.*, baking) at high temperatures, while the removal of oxygen from canned foods and beverages prevents color and flavor loss.

Glucose oxidase also is currently used to strengthen the protein complexes contained in starch-based products through increased formation of cross-links between proteins, thereby improving the quality of such foods as breads and other baked products (Vemulapalli and Hoseney, 1998; Vemulapalli *et al.*, 1998; Rasiah *et al.*, 2005). The cross-links formed may be achieved through increased formation of disulfide cross-links between amino acid residues as a result of decreasing the sulfhydryl content of the proteins through oxidation (Vemulapalli and Hoseney, 1998). Oxidation is carried out by the H_2O_2 formed as a result of glucose oxidase activity. In this instance, H_2O_2 oxidizes the cysteine residues of proteins to form cystine, thus converting the sulfhydryl groups to a disulfide bond. The mechanism of action is described by the following series of reactions:

$$\beta\text{-D-glucose} + O_2 \xrightarrow{\text{glucose oxidase}} \text{D-glucono-1,5-lactone} + H_2O_2$$

$$\underset{\text{cysteine}}{2(C_3H_6NO_2SH)} + H_2O_2 \xrightarrow{\text{oxidation}} \underset{\text{cystine}}{C_6H_{12}N_2O_4S_2} + 2H_2O$$

The proposed uses of the glucose oxidase preparation from *P. chrysogenum* strain PGO 19-162, as summarized in Table I.D-1, are consistent with those already authorized for use by the FDA or considered to be GRAS through the self-GRAS determination procedure. As mentioned in Section I.D.2, authorized uses in the U.S. consist of glucose oxidase-catalase preparations in the manufacture of dried whole eggs, dried egg whites, and dried egg yolks for the purpose of reducing the glucose content of the liquid prior to drying (21CFR §160.105, 160.145, 160.185) (U.S. FDA, 2013). Self-GRAS determinations, which have been notified to FDA, consist of a glucose oxidase preparation derived from *Aspergillus niger*, which has been determined to be GRAS for use in food in general for its technical enzymatic effect (GRAS Notice No. 000089) (U.S. FDA, 2002a). Likewise, a glucose oxidase preparation derived from *A. oryzae* carrying a gene encoding glucose oxidase from *A. niger* has been determined to be GRAS for use as an enzyme in baking applications and (in combination with a catalyze enzyme preparation) in the manufacture of foods, such as cheese, beer, carbonated beverages, and fruit juice (GRAS Notice No. 000106) (U.S. FDA, 2002b). FDA filed the GRAS notices submitted for both enzyme preparations and issued a letter of no objection in both instances.

IV.A.2 Enzyme Residues in Food

The glucose oxidase preparation is added directly to food, but the enzyme is inactivated at a temperature of 80°C [thermal instability is achieved at temperatures greater than 50°C (see Section II.G.2)], and therefore, would be inactivated under food processing conditions consisting of high temperatures, such as those which occur during baking/cooking and pasteurization/ sterilization. To confirm that the enzyme is inactivated under the proposed conditions of use and that the enzyme is not active in the final food and beverage products for consumption, analytical testing for residual glucose oxidase activity was conducted on a representative food product. The enzyme preparation with a glucose oxidase activity of 2,000 U/g was added to wheat flour at a TOS level of 3 mg/kg and the flour was added to bread dough. The dough was baked at a temperature of 200 to 250°C. Enzyme extraction was performed on 1 g of the baked bread product (in 10 mL water) and glucose oxidase activity was measured. The level of glucose activity was below the limit of detection (0.55 U/g) for the glucose oxidase assay, thus confirming the absence of enzyme activity in the final product for consumption.

IV.A.3 Estimated Daily Intake of Total Organic Solids from Intended Uses of the Enzyme Preparation

In accordance with the FDA guidance for industry on GRAS notice submissions for enzyme preparation (U.S. FDA, 2010), an estimate of the dietary exposure to the glucose oxidase preparation from *P. chrysogenum* strain PGO 19-162 was performed. The estimated daily intake of the enzyme preparation was calculated based on the level of resultant TOS in food from the addition of the enzyme preparation and the intake of those foods by the U.S. population. According to the FDA guidance, in instances where the actual TOS level in the final

food is not available, the estimated daily intake should be calculated based on the use level of the enzyme expressed on a TOS basis. Therefore, a dietary exposure assessment was performed using this approach. The maximum use level expressed as TOS was used in the assessment on the basis that the enzyme will not be removed from the proposed foods and beverages and to provide the worst case exposure scenario.

Estimates for the intake of TOS from the enzyme preparation were based on the individual food uses and use levels presented in Table I.D-1 in conjunction with food consumption data included in the U.S. National Center for Health Statistics' (NCHS) 2009-2010 National Health and Nutrition Examination Surveys (NHANES) (CDC, 2011; USDA, 2012). Food codes were grouped in food-use categories according to Title 21, Section §170.3 of the Code of Federal Regulations (U.S. FDA, 2014). Product-specific adjustment factors were developed based on data provided in the standard recipe file for the Continuing Survey of Food Intakes by Individuals (CSFII) 1994-1996, 1998 survey (USDA, 2000).

Estimates for the total daily intake of TOS from all proposed food uses of the enzyme preparation are summarized in Table IV.A.3-1 on a per person basis by population group. Table IV.A.3-2 presents these data on a per kilogram body weight basis.

Approximately 98.9% of the total U.S. population was identified as potential consumers of foods containing TOS as a result of the proposed uses of the enzyme preparation (8,195 actual users identified). For infants, 79.0% of respondents were identified as potential consumers of TOS from the enzyme preparation, while in all other population groups the percentage of consumers exceeded 99%. As a result of the high percentage of users identified within all population groups, the intake estimates for the all-person and all-user categories were similar; therefore, only the all-user results are discussed in detail.

On an all-user basis, the mean intake of TOS by the total U.S. population from all proposed food uses of the glucose oxidase preparation was estimated to be 8.5 mg/person/day. The heavy consumer (90th percentile) all-user intake of TOS by the total U.S. population from all proposed food uses was estimated to be 14.5 mg/person/day. The total population mean and 90th percentile intakes on a body weight basis were determined to be 0.14 and 0.27 mg/kg body weight/day, respectively.

When the individual population groups were examined, the largest mean and 90th percentile absolute TOS intakes were determined to occur in male adults at 10.3 and 17.4 mg/person/day, respectively. The lowest absolute mean and 90th percentile intake estimates were determined to occur in infants at 4.1 and 7.8 mg/person/day, respectively. On a body weight basis, infants were determined to have the highest mean and 90th percentile intakes at 0.34 and 0.64 mg/kg body weight/day. The mean and 90th percentile intakes in children also were high relative to the total population intakes at 0.27 and 0.48 mg/kg body weight/day, respectively. Female adults

displayed the lowest mean and 90th percentile intakes at 0.11 and 0.19 mg/kg body weight/day, respectively.

Table IV.A.3-1 Summary of the Estimated Daily Intake of Total Organic Solids from the Proposed Food Uses of Glucose Oxidase from *Penicillium chrysogenum* Strain PGO 19-162 in the U.S. by Population Group (2009-2010 NHANES Data)

Population Group	Age Group (Years)	All-Person TOS Consumption (mg/day)		All-Users TOS Consumption (mg/day)			
		Mean	90th Percentile	% Users	n	Mean	90th Percentile
Infants	0 to 2	3.3	7.1	79.0	646	4.1	7.8
Children	3 to 11	6.9	11.6	100	1,429	6.9	11.6
Female Teenagers	12 to 19	7.8	13.0	100	520	7.8	13.0
Male Teenagers	12 to 19	9.0	15.4	99.2	566	9.1	15.4
Female Adults	20 and up	7.8	12.7	99.8	2,647	7.8	12.8
Male Adults	20 and up	10.3	17.4	100	2,387	10.3	17.4
Total Population	All Ages	8.4	14.4	98.9	8,195	8.5	14.5

Table IV.A.3-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Total Organic Solids from Proposed Food Uses of Glucose Oxidase from *Penicillium chrysogenum* Strain PGO 19-162 in the U.S. by Population Group (2009-2010 NHANES Data)

Population Group	Age Group (Years)	All-Person TOS Consumption (mg/kg bw/day)		All-Users TOS Consumption (mg/kg bw/day)			
		Mean	90th Percentile	% Users	n	Mean	90th Percentile
Infants	0 to 2	0.27	0.60	79.0	646	0.34	0.64
Children	3 to 11	0.27	0.48	100	1,429	0.27	0.48
Female Teenagers	12 to 19	0.13	0.23	100	520	0.13	0.23
Male Teenagers	12 to 19	0.14	0.24	99.2	566	0.14	0.24
Female Adults	20 and up	0.11	0.19	99.8	2,647	0.11	0.19
Male Adults	20 and up	0.12	0.21	100	2,387	0.12	0.21
Total Population	All Ages	0.14	0.26	98.9	8,195	0.14	0.27

Although the highest 90th percentile all-user intake of TOS from the glucose oxidase preparation on a body weight basis was determined to be in infants, this value may not represent the most realistic intake assessment in infants. While the enzyme preparation is proposed for use in the production of foods that may be consumed by infants and toddlers, it is not intended for use in infant- or toddler-specific foods, such as infant formulas, baby foods, and toddler foods, and none of the specified food uses are intended to be marketed to infants or toddlers. As a result,

the intake of foods manufactured with the use of the glucose oxidase preparation is likely to be limited in infants and toddlers.

Furthermore, the type of methodology employed in the intake assessment is generally considered to be 'worst case' as a result of several conservative assumptions made in the consumption estimates. For example, it is often assumed that all food products within a food category contain the ingredient at the maximum specified level of use. In addition, it is well established that the length of a dietary survey affects the estimated consumption of individual users; short-term surveys, such as the typical 2- or 3-day dietary surveys, overestimate the consumption of food products that are consumed relatively infrequently. Therefore, the calculated estimated intake levels of the glucose oxidase preparation on a TOS basis are highly conservative estimates.

IV.B Information to Support the Safety of the Enzyme Preparation

The safety of the glucose oxidase preparation from *P. chrysogenum* PGO 19-162 for use in food production is assessed in the sections that follow according to the guidelines developed by Pariza and Foster (1983), Pariza and Johnson (2001), and the International Food Biotechnology Council (IFBC) (IFBC, 1990). Enzymes themselves are considered to be inherently non-toxic, and therefore, according to these guidelines, the primary consideration in evaluating enzyme safety is the safety of the production strain. The safety of a production strain is addressed primarily by evaluating its toxigenic potential; for filamentous fungi, such as *A. niger*, the oral toxins of concern are mycotoxins (small molecular weight organic molecules, usually less than 1,000 Da in size) (Pariza and Johnson, 2001). Additional considerations for evaluating the safety of a production strain include pathogenicity and antibiotic production. According to the established guidelines, the safety of the final enzyme preparation itself also should be assessed *via* analytical testing to ensure absence of toxic constituents (*e.g.*, mycotoxins) and antibiotic activity, as well as through toxicological testing to confirm the safety of the enzyme preparation where warranted.

An evaluation of the safety of the production strain *P. chrysogenum* PGO 19-162 and the glucose oxidase preparation derived from this strain is provided in the sections below based on generally available information and analytical data, while taking into consideration all elements of enzyme safety. The safety of the production strain *P. chrysogenum* PGO 19-162 is discussed throughout Section IV.B.1 as is the safety of the final glucose oxidase preparation as it relates to antibiotic activity and mycotoxins. The toxicological data confirming the safety of the enzyme preparation are summarized in Section IV.B.2 and consist of an oral subchronic toxicity study in rats and *in vitro* and *in vivo* genotoxicity data. Additional safety considerations (*i.e.*, allergenicity and production of hydrogen peroxide) are addressed in Section IV.B.3. The Pariza-Johnson decision tree outlining the safety evaluation of the glucose oxidase preparation from *P. chrysogenum* PGO 19-162 is provided in Appendix A.

IV.B.1 Safety of the Production Strain

IV.B.1.1 History of Safe Use

P. chrysogenum is a filamentous fungus with a history of safe use in Europe as a starter culture for the production of dry sausages (Sunesen and Stahnke, 2003) and in the fermentation and biopreservation of meat (Mogensen *et al.*, 2002). Suitability of *P. chrysogenum* for these uses is determined at the strain level (Sunesen and Stahnke, 2003). *P. chrysogenum* also is listed by the Association of Manufacturers and Formulators of Enzyme Products (Amfep) as a current source of a commercialized glucose oxidase enzyme preparation used in food processing (Amfep, 2009). *Penicillium* (species not specified) also is an approved source of glucose oxidase in Japan as indicated on the Ministry of Health, Labor, and Welfare's (MHLW) list of existing food additives (MHLW, 2011), and the production strain PGO 19-162 has been used in Japan in the commercial production of Shin Nihon's glucose oxidase preparation described herein. The glucose oxidase preparation from *P. chrysogenum* PGO 19-162 has been commercially marketed in Japan since 1999, and has been used in the production of foods, such as baked goods, cooked rice, and mayonnaise. Shin Nihon's glucose oxidase preparation therefore has a history of safe use in food production in Japan. The safety assessment of *P. chrysogenum* PGO 19-162 is presented below, with emphasis on the toxicogenic potential and antibiotic production of the strain. The pathogenicity of the strain also is discussed.

IV.B.1.2 Toxigenicity

The main concern when assessing the safety of a production strain is its toxigenic potential. The oral toxins of concern associated with filamentous fungi are the secondary metabolites classified as mycotoxins (Pariza and Johnson, 2001). *P. chrysogenum* has been reported to produce the mycotoxins roquefortine C, PR toxin, and secalonic acids (Frisvad *et al.*, 2004). Consistent with this literature, an analysis of the secondary metabolite profile of *P. chrysogenum* PGO 19-162 revealed production of roquefortine C. Roquefortine C analyses of the glucose oxidase enzyme preparation, however, demonstrate absence of this mycotoxin in the final enzyme preparation (see Section II.F.2). No other mycotoxins, including PR toxin and secalonic acids, are produced by *P. chrysogenum* PGO 19-162. The final enzyme preparations therefore do not contain detectable levels of mycotoxins.

That the production strain does not produce the major food-contaminating mycotoxins of concern to food safety is consistent with the secondary metabolite profile reported for this species (Frisvad *et al.*, 2004) and other literature. For instance, while ochratoxin A is produced by some species of the *Penicillium* genus (Moss, 2002; Murphy *et al.*, 2006), only *P. nordicum* and *P. verrucosum* are known to produce ochratoxins (Frisvad *et al.*, 2004). Moreover, aflatoxins are produced only by *Aspergillus*, mainly strains belonging to the species *A. flavus* and *A. parasiticus* (Bennett and Klich, 2003), zearalenone and T-2 toxin are produced only by

Fusarium species (Bennett and Klich, 2003), and sterigmatocstin is not produced by *Penicillium* (Frisvad *et al.*, 2004; Rank *et al.,* 2011).

Other mycotoxins of concern produced by *Penicillium* species include patulin, cyclopiazonic acid, and penicillic acid (Moss, 2002; Frisvad *et al.*, 2004; Murphy *et al.*, 2006); however, these have not been identified as secondary metabolites produced by *P. chrysogenum* (Frisvad and Filtenborg, 1983; Frisvad *et al.*, 2004), including strain PGO 19-162.

P. chrysogenum has been reported to produce the secondary metabolite chrysogine (Frisvad *et al.*, 2004), and the secondary metabolite analysis of *P. chrysogenum* PGO 19-162 indicates that the production strain produces this metabolite. Chrysogine is an anti-auxin, which is a substance that has an inhibitory effect on a class of plant hormones known as auxins. The compound is not known to have potential pharmaceutical or mycotoxin activity (Frisvad *et al*., 2004). Although standards for this compound are not readily available for analytical testing, the mycotoxin results for the enzyme preparation indicate that the purification steps applied in the manufacture of the glucose oxidase preparation are sufficient in removing any toxins of concern. That no oral toxins are present in the final enzyme preparation is supported by the results of the product-specific oral subchronic toxicity study conducted in rats (summarized in Section IV.B.2.1), which demonstrate that no compound-related adverse effects were observed in rats at doses of up to 193 mg TOS/kg body weight/day, the highest dose tested.

Based on the information provided above and the history of safe use of the enzyme preparation, the glucose oxidase preparation from *P. chrysogenum* PGO 19-162 is considered to be free of detectable levels of toxic metabolites. This conclusion is supported by the results of the oral subchronic toxicity study summarized in Section IV.B.2.1, which demonstrate that the enzyme preparation does not result in systemic toxicity in rats following oral exposure at doses far in excess of the estimated daily intakes.

IV.B.1.3 Pathogenicity

With regards to the pathogenicity of *P. chrysogenum*, some Penicillium species have been reported to occasionally cause opportunistic infections in humans, and in particular, as fungal etiological agents in fungemia, pneumonia, peritonitis, urinary tract infections, endocarditis, and disseminated infections (Swoboda-Kopec *et al.*, 2003). Invasive *Penicillium* infections, however, are rare and the majority of these is not acquired under normal conditions, but are associated with conditions which would render an individual susceptible to infection, including injury, surgery and other invasive procedures, and use of prosthetic materials (Lyratzopoulos *et al.*, 2002). Likewise, infections caused specifically by the species *P. chrysogenum* are extremely rare. Although several case reports of infection caused by *P. chrysogenum* have been identified in the scientific literature, such cases consisted of opportunistic infections occurring in patient population groups, including in individuals with compromised immune systems [*e.g.,* acquired immunodeficiency syndrome (AIDs), undergoing chemotherapy for

acute myeloid leukemia, valve surgery)] and in those having endured injury (*i.e.,* incidental skull/scalp trauma) or surgery (Yassin *et al.*, 1978; Eschete *et al.*, 1981; Prasad and Nema, 1982; Hoffman *et al.*, 1992; D'Antonio *et al*., 1997; Keung *et al.,* 1997; Lyratzopoulos *et al.*, 2002; Swoboda-Kopec *et al.*, 2003; Barcus *et al.,* 2005). Only 2 cases of infection in non-immunocompromised individuals were identified in the available literature (López-Martínez *et al.*, 1999; Kantarcioğlu *et al.*, 2004). Strains of *P. chrysogenum* are thus generally considered to have no or little pathogenicity (Botterel *et al.*, 2002; Kantarcioğlu *et al.*, 2004; Barcus *et al.*, 2005). The production strain *P. chrysogenum* is therefore considered to be non-pathogenic.

IV.B.1.4 Antibiotic Production

P. chrysogenum is a well-known producer of β-lactam antibiotics, including the antibiotics meleagrin, xanthocillin, sorrentanone, and sorbicillin (Frisvad *et al.*, 2004). Consistent with this information, the secondary metabolite analysis of *P. chrysogenum* PGO 19-162 indicates that the production strain produces the antibiotics meleagrin and xanthocillin X. As a result, to determine whether *P. chrysogenum* PGO 19-162 produces antibiotic activity, the strain was tested using the antibacterial activity assay established by JECFA (2006b). The analytical results demonstrate that the production strain does not produce any detectable levels of antibacterial activity. Additionally, the product specifications for the final glucose oxidase preparations ensure that no antibacterial activity is present in the final enzyme preparations. The analytical data demonstrating that the final glucose oxidase preparations lack antibacterial activity are presented in Section II.E.

IV.B.2 Toxicity Studies on the Enzyme Preparation

The toxicity of the glucose oxidase preparation from *P. chrysogenum* PGO 19-162 was assessed by Konishi *et al*. (2013) in a battery of classical toxicity tests, consisting of a repeated-dose 90-day oral toxicity study conducted in rats and a series of mutagenicity and genotoxicity tests, including a bacterial reverse mutation test, an *in vitro* mammalian chromosomal aberration test, and a combined *in vivo* mammalian erythrocyte micronucleus test and comet assay. All tests were performed in compliance with the Organisation of Economic Co-operation and Development (OECD) Principles of Good Laboratory Practice (GLP) (OECD, 1998a) and in accordance with the OECD Guidelines for the Testing of Chemicals where applicable. The studies are summarized in the sections that follow and comprise the pivotal data supporting the safety of the glucose oxidase preparation from *P. chrysogenum* PGO 19-162.

IV.B.2.1 Subchronic Toxicity

The subchronic oral toxicity of the glucose oxidase preparation from *P. chrysogenum* PGO 19-162 was assessed in a 90-day toxicity study conducted in rats, which was performed in accordance with OECD Test No. 408 (OECD, 1998b) (Konishi *et al*., 2013). The doses administered in the study were selected based on the results of a 14-day dose-range finding

study in which no compound-related adverse effects were observed in Crl:CD(SD) rats (5 animals/sex/group) at doses providing 156 (low-dose), 1,560 (mid-dose), and 15,600 (high-dose) U/kg body weight/day administered by gavage. The corresponding doses on a TOS basis were 93, 19.3, and 193 mg TOS/kg body weight/day in the low-, mid-, and high-dose groups, respectively. A group of 5 male and 5 female control animals were also administered the vehicle control (distilled water). Based on the results of the dose-range finding study, 15,600 (high-dose) U/kg body weight/day was selected as the high-dose to be administered in the 90-day study.

In the subchronic study, groups of 10 male and 10 female Crl:CD(SD) rats were orally administered the enzyme preparation by gavage at doses providing 156 (low-dose), 1,560 (mid-dose), or 15,600 (high-dose) U/kg body weight/day for 90 days (Konishi *et al*., 2013). The corresponding doses on a TOS basis were 93, 19.3, and 193 mg TOS/kg body weight/day in the low-, mid-, and high-dose groups, respectively. The control group consisted of 10 males and 10 females administered the vehicle control (water). All animals were observed twice daily for clinical signs, and individual body weights and food consumption were measured weekly. Ophthalmological examinations were conducted in control and high-dose animals prior to study initiation and on day 83. Fasting blood samples were obtained from all animals on days 91 or 92 for routine hematological, blood coagulation, and serum chemistry analyses. Urine samples were obtained on day 85 following overnight fasting and collection for urinalysis. All animals were euthanized on the day after the last dose administration. Complete necropsies were conducted on all animals, and their organs were removed and weighed. Histopathological examination of organs and tissues was conducted for all animals in the control and high-dose groups.

No mortalities were observed during the 90-day study period, and no compound-related clinical signs or ophthalmological abnormalities were observed. In addition, no significant differences in body weight, body weight gain, or food consumption were observed between the control group and any of the glucose oxidase-administered dose groups throughout the course of the study.

No significant differences in any of the blood coagulation parameters were observed between the control and glucose oxidase-administered dose groups. Statistically significant differences in hematology parameters observed in the glucose oxidase groups compared to the control group included the following: increased white blood cell count, neutrophil count, and large unstained cell count in low-dose females and decreased platelet count in mid-dose males. In addition, clinical chemistry analysis revealed significantly increased γ-globulin ratio and concentration in low-dose females, as well as significantly increased blood glucose levels in high-dose males. The hematology and clinical chemistry variations observed in the low- and mid-dose groups were not observed at higher dose levels (*i.e.*, changes were non-dose-dependent) and were not consistent among sexes. Moreover, although blood glucose levels were increased in males of the high-dose group, this value (167 ± 13 mg/dL) remained within

the range of historical background data (154 ± 26 mg/dL, n=70). Furthermore, none of the changes observed were associated with compound-related histopathological abnormalities, and therefore, the variations observed were considered to be incidental and not compound-related or toxicologically significant.

Several statistically significant differences in urinary electrolyte parameters were observed in the glucose oxidase groups compared to the control group and included the following changes: increased urinary sodium concentration, total sodium excretion, and total potassium excretion in high-dose males and increased urinary potassium concentration in high-dose females. In addition, a significant non-dose-dependent decrease in urinary chloride concentration was observed in mid- and high-dose females compared to control females. This latter effect was associated with a significant decrease in total chloride excretion in these groups, which appeared to be dose-dependent. All urinary electrolyte changes, however, were small in magnitude and did not result in any effects on urinary volume or blood electrolyte concentrations and were not accompanied by any clinical findings which might lead to changes in electrolyte levels. Furthermore, changes in urinary electrolyte parameters were not accompanied by changes in clinical chemistry indices that would suggest renal toxicity (*i.e.*, BUN and creatinine concentrations) or any compound-related gross (including changes in kidney weights) or histopathological manifestation of nephropathy. Therefore, the urinary electrolyte changes observed were not considered to be toxicologically significant.

Absolute seminal vesicle weights in low-dose males were significantly increased compared to controls; however, this effect was not considered to be compound-related due to the lack of a dose-response relationship and no effects on relative seminal vesicle weights. Relative thymus weights of high-dose males were significantly decreased compared to controls; however, the investigators noted that this reduction was an artifact of increased thymus weights in the control group (0.073 ± 0.018%) as determined from a review of the historical control data (0.06 ± 0.04%, n=115). Moreover, the relative thymus weights in all glucose oxidase groups were within the range of historical background data (low-dose = 0.059 ± 0.013%, mid-dose = 0.060 ± 0.008%, high-dose = 0.058 ± 0.010%). Therefore, the variation in thymus weight was not considered to be compound-related or toxicologically relevant. No other significant differences in absolute or relative organ weights were observed among groups. Furthermore, no compound-related macroscopic or histopathological findings were observed.

Based on the results of this study, the no-observed-adverse-effect level (NOAEL) for the oral toxicity of the glucose oxidase preparation from *P. chrysogenum* PGO 19-162 was determined to be 15,600 U/kg body weight/day in rats, equivalent to 193 mg TOS/kg body weight/day, the highest dose tested. In comparison, the estimated 90th percentile daily intake of TOS from the use of the glucose oxidase preparation from *P. chrysogenum* PGO 19-162 in the total U.S. population was calculated to be 0.27 mg/kg body weight/day. The greatest estimated daily intakes calculated among all population groups on a body weight basis occurred in infants and

children. Estimated 90th percentile daily intakes in these population groups were calculated to be 0.64 and 0.48 mg/kg body weight/day. Thus, a large margin of safety exists between the NOAEL for the glucose oxidase preparation and the estimated 90th percentile daily intakes on a TOS basis. No safety concerns are therefore raised with respect to the conditions of use of the glucose oxidase preparation from *P. chrysogenum* PGO 19-162.

IV.B.2.2 Mutagenicity and Genotoxicity

In vitro

The potential mutagenicity of the glucose oxidase preparation from *P. chrysogenum* PGO 19-162 was evaluated in the bacterial reverse mutation test (Ames test) performed according to OECD Test No. 471 (OECD, 1997a) (Konishi *et al*., 2013). The test was conducted in *Salmonella typhimurium* TA100, TA98, TA1535, and TA1537 and *Escherichia coli* WP2*uvr*A in the presence and absence of S9 metabolic activation. For the purposes of this test, the glucose oxidase enzyme was rendered inactive by heating to a temperature of 60°C and adjusting the pH to 2. The pH was then re-adjusted to the original pH of the test substance (approximately 4). The enzyme was inactivated on the basis that the reaction catalyzed by glucose oxidase produces hydrogen peroxide as a by-product. Hydrogen peroxide is a well-known reactive oxygen species, which may cause cellular and sub-cellular oxidative damage, including damage to DNA. Thus, to ensure that secondary genetic mutations arising from potential hydrogen peroxide production would not occur, the enzyme was inactivated. A reference control [vehicle control (water) treated in the same manner as the test article during pH adjustment] was included to control for the pH adjustment treatment, and all test results were compared to the value obtained with the reference control. Appropriate positive controls also were included. A preliminary concentration-range finding test and a main test were performed. Both tests were conducted in triplicate at final glucose oxidase concentrations of 0.493, 1.56, 4.93, 15.6, 49.3, and 156 U/plate in the presence and absence of S9 metabolic activation. The corresponding concentrations on a TOS basis were 0.00611, 0.0193 0.0611, 0.193, 0.611, and 1.93 mg TOS/plate, respectively. In both the concentration-range finding test and the main test, no positive mutagenic responses (*i.e.,* reproducible 2-fold or dose-dependent induction in the number of revertant colonies compared to the reference control) were observed in any strain at any concentration tested in either the presence or absence of metabolic activation. In contrast, positive control substances displayed marked mutagenic activity. Based on the results of this study, the investigators concluded that the glucose oxidase preparation from *P. chrysogenum* PGO 19-162 was non-mutagenic in the bacterial reverse mutation test.

The clastogenic potential of the glucose oxidase preparation from *P. chrysogenum* PGO 19-162 was investigated in an *in vitro* chromosomal aberration test conducted in cultured human lymphocytes in accordance with OECD Test No. 473 (OECD, 1997b), using both the short-term (3-hour) and continuous (24-hour) treatment methods (Konishi *et al*., 2013). The short-term

assay was conducted in the presence and absence of S9 metabolic activation and the continuous assay was conducted in the absence of metabolic activation. The glucose oxidase enzyme was not inactivated for the purposes of this test. Distilled water served as the negative control. Mitomycin C was used as the positive control in assays conducted in the absence of metabolic activation and cyclophosphamide was used as the positive control in the presence of metabolic activation. All control and treatments were conducted in duplicate. In the short-term assays, human lymphocytes were incubated with the test article at concentrations of 1.22, 2.44, 4.88, 9.75, 19.5, or 39.0 U/mL, corresponding to concentrations of 0.0151, 0.0303, 0.0605, 0.121, 0.242, 0.484 mg TOS/mL, respectively. In the 24-hour continuous treatment assay, lymphocytes were incubated with the test article at concentrations of 0.00952, 0.0190, 0.0381, 0.0762, 0.152, or 0.305 U/mL, corresponding to concentrations of 0.000118, 0.000236, 0.000472, 0.000945, 0.00188, and 0.00378 mg TOS/mL, respectively. The lowest test article concentration resulting in a relative mitotic index (MI) of less than 50% was 19.5 U/mL under short-term treatment with and without S9 metabolic activation and 0.0762 U/mL under continuous treatment. Thus, the test article concentrations that were assessed for structural chromosome aberrations were 4.88, 9.75, and 19.5 U/mL in the short-term assays and 0.0190, 0.0381, and 0.0762 U/mL in the continuous assay. A concentration-dependent decrease in the relative MI was observed at concentrations of 4.88, 9.75, and 19.5 U/mL in both short-term assays and at concentrations of 0.0190, 0.0381, and 0.0762 U/mL in the continuous assay. In all assays, no statistically significant differences in the incidence of cells with structural chromosomal aberrations were observed in cells treated with the glucose oxidase preparation compared to the negative control. In addition, the incidence of polyploidy cells in cells treated with the glucose oxidase preparation was not significantly different from that of the negative control group in all assays. In contrast, treatment with positive control agents produced a significant increase in the percentage of cells with structural chromosome aberrations compared to the negative control. Based on the results of this study, it may be concluded that the glucose oxidase preparation from *P. chrysogenum* PGO 19-162 is non-clastogenic in human lymphocytes.

In vivo

The genotoxic potential of the glucose oxidase preparation from *P. chrysogenum* PGO 19-162 was further investigated in a combined *in vivo* mammalian erythrocyte micronucleus test and comet assay conducted in rats (Konishi *et al*., 2013). The mammalian erythrocyte micronucleus test portion of this study was conducted in accordance with OECD Test No. 474 (OECD., 1997c). Groups of 5 male Crl:CD(SD)[SPF] rats were orally administered the glucose oxidase preparation at doses providing 3,900 (low-dose), 7,800 (mid-dose), or 15,600 (high-dose) U/kg body weight/day for 3 consecutive days. The corresponding doses on a TOS basis were 48.5, 96.7, and 193 mg TOS/kg body weight/day in the low-, mid-, and high-dose groups, respectively. A negative control group received 10 mL/kg body weight/day of the vehicle control (distilled water), and a positive control group received 200 mg/kg body weight/day of ethyl

methanesulfonate (EMS). Body weights were measured prior to the dosing period and before necropsy. All animals were observed for clinical signs of toxicity once daily after dosing and prior to necropsy. All rats were euthanized 3 hours following the final dose, and their glandular stomach, liver, and femur were removed. Bone marrow samples were obtained from the femur and assessed in the micronucleus assay. The stomach and liver were examined macroscopically, and cell suspensions prepared from these organs were examined for DNA damage in the comet assay.

No clinical signs of toxicity or adverse effects on body weight gain were observed in the glucose oxidase groups. In addition, there were no macroscopic findings observed in the liver or stomach of all animals. In the micronucleus test, there were no significant differences in the frequency of micronucleated cells in the glucose oxidase groups compared to the negative control group. A significant increase in the ratio of immature erythrocytes to the total number of analyzed erythrocytes was observed in the mid-dose group; however, this finding is not considered to be toxicologically significant due to the absence of a dose-response relationship. In the comet assay, no evidence of increased DNA damage was observed in liver or stomach cells of rats administered the glucose oxidase preparation compared to the negative control. Administration of EMS resulted in significant increases in the frequency of micronucleated cells in the micronucleus test and DNA damage in the comet assay compared to the negative control. Based on their findings, the investigators concluded that the glucose oxidase preparation from *P. chrysogenum* PGO 19-162 was non-genotoxic *in vivo* in the mammalian erythrocyte micronucleus test and the comet assay.

IV.B.3 Additional Safety Considerations

IV.B.3.1 Allergenicity

Pariza and Foster (1983) have reported that "*Allergies and primary irritations from enzymes used in food processing should be considered a low priority item of concern except in very unusual circumstances.*" To confirm that glucose oxidase produced by *P. chrysogenum* PGO 19-162 does not contain amino acid sequences similar to known allergens that might produce an allergenic response, a sequence homology search was conducted using the AllergenOnline database version 13 (available at http://www.allergenonline.org; updated February 12, 2013) maintained by the Food Allergy Research and Resource Program of the University of Nebraska. The database contains a comprehensive list of putative allergenic proteins developed *via* a peer reviewed process for the purpose of evaluating food safety (FARRP, 2013a). A full-length alignment search of AllergenOnline was conducted using default settings (*E* value cutoff = 1 and maximum alignments of 20) and the results are presented in Table IV.B.3.1-1. A high degree of

similarity, as indicated by the E value[2], (E value = 2.7 e-021) was identified between the full length *P. chrysogenum* PGO 19-162 glucose oxidase amino acid sequence and the Mala s 12 allergen precursor produced by the fungal species *Malassezia sympodialis*. The percent identity[3] of the amino acid sequences however was low at 31%, indicating a low potential for cross-reactivity to this allergen. Other matches identified were considered to be poor matches and consisted of matches to putative allergens produced by common ragweed (*Ambrosia artemisiifolia*), with none sharing a percent identity of greater than 50% over the length of the glucose oxidase sequence, which indicates that the potential for cross-reactivity to these allergens is low. Additionally, no matches to any of the 8 major food allergens were identified, the 8 major food allergens being 1 of the following foods and ingredients that contain protein from them: milk, egg, peanuts, tree nuts, soybeans, wheat, fish, and Crustacean shellfish as stipulated under the Food Allergen Labeling and Consumer Protection Act of 2004 (U.S. FDA, 2004).

Table IV.B.3.1-1 Overall Search of AllergenOnline Database Version 13 with the Full Length Glucose Oxidase Amino Acid Sequence from *Penicillium chrysogenum* PGO 19-162[a]

Sequence G.I. #	Organism	Description	Length	*E* Value	% Identity	Amino acid Alignment
78038796	*Malassezia sympodialis*	Mala s 12 allergen precursor	681	2.7 e-021	31.0	584
302127820	*Ambrosia artemisiifolia*	Putative pectate lyase precursor	397	0.61	31.0	71
302127816	*Ambrosia artemisiifolia*	Putative pectate lyase precursor	397	0.61	31.0	71
113477	*Ambrosia artemisiifolia*	Pollen allergen Amb a 1.3	397	0.61	31.0	71
302127822	*Ambrosia artemisiifolia*	Putative pectate lyase precursor	397	0.61	31.0	71
302127818	*Ambrosia artemisiifolia*	Putative pectate lyase precursor	397	0.61	31.0	71
302127814	*Ambrosia artemisiifolia*	Putative pectate lyase precursor	397	0.61	31.0	71
166443	*Ambrosia artemisiifolia*	Antigen E	397	0.61	31.0	71

[a] The search was performed on June 17, 2013.

A second homology search was conducted according to the approach outlined by the Food and Agriculture Organization of the United Nations (FAO), World Health Organization (WHO)

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

[3] Proteins with greater than 70% identical primary amino acid sequences throughout the length of the protein compared to an allergen are commonly cross-reactive, while those with less than 50% identity are rarely cross-reactive (Aalberse, 2000).

(FAO/WHO, 2001) and the Codex Alimentarius Commission (2003a). In accordance with this guideline, the AllergenOnline database was searched using a sliding window of 80-amino acid sequences (segments 1-80, 2-81, 3-82, *etc*.) derived from the full- length glucose oxidase amino acid sequence. The 80 amino acid alignment search was conducted using default settings (*E* value cutoff = 1 and maximum alignments of 20). According to the approach adopted by the Codex Alimentarius Commission, significant homology is defined as an identity match of greater than 35%, and in such instances, cross-reactivity with the known allergen should be considered a possibility. The 35% identity for 80 amino acid segments is a suggested guideline proposed by the Codex Alimentarius Commission for evaluating newly expressed proteins produced by recombinant-DNA microorganisms (Codex Alimentarius Commission, 2003a), and is the same approach proposed for genetically modified crops (Codex Alimentarius Commission, 2003b). Using this search strategy, one identity match of 45% to the Mala s 12 allergen precursor produced by *Malassezia sympodialis* was identified (see Tables IV.B.3.1-2 and IV.B.3.1-3), consistent with the results of the full-length alignment search. However, Goodman *et al.* (2005) and Goodman and Hefle (2005) discuss this approach of evaluating segments of 80 amino acids to be quite conservative and precautionary. On a similar note, Aalberse (2000) notes that proteins with less than 50% identity over the length of the proteins are rarely cross-reactive; a 70% identity is typically required for cross-reactivity. Therefore, considering that the percent identity of the full length amino acid sequences was low at 31%, the potential for cross-reactivity to the Mala s 12 allergen precursor is low.

Table IV.B.3.1-2 80 mer Sliding Window Search Results for Glucose Oxidase Amino Acid Sequence from *Penicillium chrysogenum* PGO 19-162[a]

Database	AllergenOnline Database v13 (February 12, 2013)

Input Query	`>query`
	`YSPAEQIDVQSHLLSDPTKVEGETYDYVIAGGGLTGLTVAAKLSENPKIKVL`
	`VIEKGFYESNDGPIIEDPNAYGEIFGTSVDQNYLTVPLINNRTGEIKSGLGL`
	`GGSTLINGDSWTRPDKVQIDSWEKVFGMEGWNWDNVFQYMQKAERSRPPTAA`
	`QIEAGHFYDPACHGTDGTVHAGPRDNGKPWSPLMRALMNTVSAFGVPVQKDF`
	`HCGHPRGVSMIPNNLHENQIRADAAREWLLPNQRDNLQILTGQKVGKVLFNQ`
	`TASGPKAVGVNFGTNKAVNFNVYAKQEVLLAAGSAISPLILEYSGIGIKSVL`
	`DKAGVKQLLELPVGLNMQDQTTTTVRSRANNAPGQGQAAYFANFTEVLGDHA`
	`AQGIKLLDTKLDQWAEETVARGGFHNVTALKIQYENYRNWLLDEDVAFAELF`
	`FDTEGKINFDIWNLIPFTRGSVHILSSDPYLWQYANDPKFFMNELDLLGQAA`
	`ATKLGRELSSAGEMKKYYAGETIPGDNLPQDATVEQWEDYVMMNFRPNWHAV`
	`STCSMMSRELGGVVDATAKVYGTQGLRVIDGSIPPTQVSSHVMTVFYGMALR`
	`IAESVLEDYAKKA`

Length	586
Number of 80 mers	507
Number of Sequences with hits	1

[a] The search was performed on June 17, 2013.

Table IV.B.3.1-3 80 mer Sliding Window Search Results for Glucose Oxidase Amino Acid Sequence from *Penicillium chrysogenum* PGO 19-162[a]

Defline	Species	Best % Identity	Number of Hits >35%	Full Alignment		
				E value	% Identity	Length
gi|78038796|emb|CAI43283.4| mala s 12 allergen pre	*Malassezia sympodialis*	45.00%	94 of 507	2.7 e-021	31.00%	584

The Codex Alimentarius Commission further notes that cross-reactivity with a known allergen may need to be considered when 6 contiguous amino acids are identified [although, there are no known examples where an isolated identity match of 6 or 8 amino acids was found between cross-reactive proteins unless there was at least a 35% identity match over 80 amino acids (Goodman *et al.*, 2008)]. The AllergenOnline sequence search tools do not include a function to search for 6 contiguous amino acids, but do include a function for searching for 8 contiguous amino acids. Using this tool, no exact matches were identified.

Based on the information provided above, no evidence exists that might indicate that the glucose oxidase produced by *P. chrysogenum* PGO 19-162 would produce an allergenic response following consumption of foods to which the enzyme has been added. Additionally, there is no evidence from the available scientific literature or from the history of use of the enzyme preparation in Japan indicating allergenicity to glucose oxidase in consumers of foods to which the enzyme has been added. Furthermore, the enzyme would be inactivated and denatured under the conditions of food processing, and therefore, would not have the potential to result in allergenicity in consumers.

With regards to the use of soybean meal, one of the major food allergens, as a component of the fermentation media used in the production of the glucose oxidase enzyme, it is not anticipated that soybean protein would be present in the final enzyme preparation; following fermentation, the fermentation media is separated and purified from the enzyme during the recovery process. There are therefore no allergenicity concerns with the use of soybean meal as a component of the fermentation media used in the production of the glucose oxidase enzyme. This is consistent with generally available information on the potential allergenicity of microbially-derived enzyme preparations from the use of certain fermentation media raw materials as summarized in the Enzyme Technical Association (ETA) position paper *Food Allergen Labeling of Microbially Derived Enzymes under FALCFA as it Applies to Fermentation Media Raw Materials* (ETA, 2005) and the expert opinion statement of the Food Allergy Research and Resource Program at the University of Nebraska *Testing of Microbially Derived Enzymes for Potential Allergens from Fermentation Media Raw Materials* (Food Allergy Research and Resource Program, University of Nebraska - FARRP, 2013b).

IV.B.3.2 Hydrogen Peroxide Production

As mentioned, hydrogen peroxide is produced as a normal by-product of the reaction catalyzed by glucose oxidase. The level of hydrogen peroxide that would be produced under the conditions of use of the glucose oxidase preparation from *P. chrysogenum* PGO 19-162 would be congruent to the levels produced by the current uses of glucose oxidase derived from other sources, which are considered safe. In addition, the enzyme preparation will be used only at the minimum level necessary to achieve the desired intended effect, limiting any excessive production of hydrogen peroxide. On this basis, no safety concerns are raised with respect to the production of hydrogen peroxide from the use of the glucose oxidase preparation from *P. chrysogenum* PGO 19-162 as a processing aid.

IV.C Expert Panel Evaluation

Shin Nihon Chemical Co., Ltd. has determined that the glucose oxidase preparation from *Penicillium chrysogenum* strain PGO 19-162 is GRAS for use as a processing aid in the production of food and beverage products as described in Table I.D.2-1 on the basis of scientific procedures. This GRAS determination is based on data generally available in the public domain pertaining to the safety of the glucose oxidase preparation, as discussed herein, and on consensus among a panel of experts (the Expert Panel) who are qualified by scientific training and experience to evaluate the safety of food ingredients. The Expert Panel consisted of the following qualified scientific experts: Dr. Joseph F. Borzelleca (Virginia Commonwealth University School of Medicine), Dr. Michael W. Pariza (Michael W. Pariza Consulting LLC), and Dr. Stanley M. Tarka, Jr. (The Tarka Group, Inc.).

The Expert Panel, convened by Shin Nihon Chemical Co., Ltd., independently and critically evaluated all data and information presented herein, and also concluded that the glucose oxidase preparation from *Penicillium chrysogenum* strain PGO 19-162 was GRAS for use as a processing aid in the production of food and beverage products as described in Table I.D.2-1 based on scientific procedures. A summary of data and information reviewed by the Expert Panel, and evaluation of such data as it pertains to the proposed GRAS uses of the glucose oxidase preparation from *Penicillium chrysogenum* strain PGO 19-162 is presented in Appendix A.

IV.D Conclusion

Based on the above data and information presented herein, Shin Nihon Chemical Co., Ltd. has concluded that the intended food uses of the glucose oxidase preparation from *Penicillium chrysogenum* strain PGO 19-162, as described in Section I.D, is GRAS based on scientific procedures. General recognition of Shin Nihon Chemical Co., Ltd.'s GRAS determination is supported by the unanimous consensus rendered by an independent Panel of Experts, qualified by experience and scientific training, to evaluate the use of the glucose oxidase preparation

from *Penicillium chrysogenum* strain PGO 19-162 in food, who similarly concluded that the intended use of the glucose oxidase preparation from *Penicillium chrysogenum* strain PGO 19-162 described herein is GRAS.

The glucose oxidase preparation from *Penicillium chrysogenum* strain PGO 19-162 therefore may be marketed and sold for its intended purpose in the U.S. without the promulgation of a food additive regulation under Title 21 of the CFR.

V REFERENCES

Aalberse RC (2000). Structural biology of allergens. J Allergy Clin Immunol 106(2):228-238.

Amfep (2009). *List of Enzymes October 2009*. Brussels, Belgium: Association of Manufacturers & Formulators of Enzmye Products (Amfep). Available at: http://www.amfep.org/content/list-enzymes.

AOAC (2000). AOAC Official Method 989.13. Motile *Salmonella* in all foods. *Official Methods of Analysis of the Association of Official Analytical Chemists: Vols. 1&2, 17th edition* (2002, Revision 1). Arlington (VA): Association of Official Analytical Chemists (AOAC).

Bankar SB, Bule MV, Singhal RS, Ananthanarayan L (2009). Glucose oxidase--an overview. Biotechnol Adv 27(4):489-501.

Barcus AL, Burdette SD, Herchline TE (2005). Intestinal invasion and disseminated disease associated with *Penicillium chrysogenum*. Ann Clin Microbiol Antimicrob 4:21. doi:10.1186/1476-0711-4-21.

Bennett JW, Klich M (2003). Mycotoxins. Clin Microbiol Rev 16(3):497-516.

Botterel F, Cordonnier C, Barbier V, Wingerstmann L, Liance M, Coste A *et al.* (2002). Aspergillus fumigatus causes in vitro electrophysiological and morphological modifications in human nasal epithelial cells. Histol Histopathol 17(4):1095-1101.

CDC (2011). *National Health and Nutrition Examination Survey (NHANES): 2009-2010*. Hyattsville (MD): Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). Available at: http://www.cdc.gov/nchs/nhanes/nhanes2009-2010/nhanes09_10.htm [Page last updated: November 7, 2011].

Codex Alimentarius Commission (2003a). *Report of the Fourth Session of the Codex Ad hoc Intergovernmental Task Force on Foods Derived from Biotechnology* (Yokohama, Japan 11-14 March 2003). (ALINORM 03/34A). Twenty-sixth Session, June 30 - July 7 2003. Rome, Italy: Food and Agriculture Organization of the United Nations (FAO), World Health Organization (WHO), Codex Alimentarius Commission (CODEX). Available at: ftp://ftp.fao.org/docrep/fao/meeting/006/y9220e.pdf.

Codex Alimentarius Commission (2003b). *Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant DNA Plants*. (CODEX-STAN CAC/GL 45-2003). Rome, Italy: Codex Alimentarius Commission (CODEX-STAN). Available at: http://www.who.int/foodsafety/biotech/en/codex_guidelines_plants.pdf.

Crueger A, Crueger W (1990). Glucose transforming enzymes. In: Fogarty WM, Kelly CT, editors. *Microbial Enzymes and Biotechnology, 2nd edition*. London, Engl. / New York (NY): Elsevier Applied Science, pp. 177-226.

D'Antonio D, Violante B, Farina C, Sacco R, Angelucci D, Masciulli M *et al.* (1997). Necrotizing pneumonia caused by Penicillium chrysogenum. J Clin Microbiol 35(12):3335-3337.

Eschete ML, King JW, West BC, Oberle A (1981). Penicillium chrysogenum endophthalmitis. Mycopathologia 74(2):125-127.

ETA (2005). *Position Paper: ETA Position on Food Allergen Labeling of Microbially Derived Enzymes Under FALCPA as it Applies to Fermentation Media Raw Materials*. (DC-749346 v1). Washington (DC): Enzyme Technical Association (ETA). Available at: http://www.enzymeassociation.org/wp-content/uploads/2013/09/Allergen-psn-paper-2.pdf [provided to FDA on September 12, 2005].

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. Rome, Italy: Food and Agriculture Organization of the United Nations (FAO), World Health Organization (WHO). Available at: ftp://ftp.fao.org/es/esn/food/allergygm.pdf.

FARRP (2013a). *AllergenOnline Version 13: Home of the FARRP Allergen Protein Database*. Lincoln (NB): University of Nebraska-Lincoln, Food Allergy Research and Resource Program (FARRP). Available at: http://farrp.unl.edu/ ; http://www.allergenonline.org/ [February 12, 2013].

FARRP (2013b). *Testing of Microbially Derived Enzymes for Potential Allergens from Fermentation Media Raw Materials*. (Expert Opinion Statement prepared by Steve L. Taylor and Joe L. Baumert with assistance from Enzyme Technical Association). Lincoln (NB): University of Nebraska-Lincoln, Food Allergy Research and Resource Program (FARRP). Available at: http://farrp.unl.edu/resources/gi-fas/opinion-and-summaries/microbiallyderivedenzymesa [August 13, 2013].

FCC (2012). Enzyme preparations. In: *Food Chemicals Codex, 8th edition*. Rockville (MD): United States Pharmacopeial Convention (USP), pp. 375-380.

Frisvad JC, Filtenborg O (1983). Classification of terverticillate penicillia based on profiles of mycotoxins and other secondary metabolites. Appl Environ Microbiol 46(6):1301-1310.

Frisvad JC, Smedsgaard J, Larsen TO, Samson RA (2004). Mycotoxins, drugs and other extrolites produced by species in *Penicillium* subgenus *Penicillium.* Stud Mycol 49:201-241.

FSIS/USDA (2013). Part 590—Inspection of eggs and egg products (Egg Products Inspection Act). Section §590.5—Terms defined". In: *U.S. Code of Federal Regulations (CFR). Title 9: Animals and Animal Products (Food Safety and Inspection Service, US Department of Agriculture).* Washington (DC): U.S. Government Printing Office (GPO). Available at: http://www.gpo.gov/fdsys/browse/collectionCfr.action?collectionCode=CFR.

Goodman RE, Hefle SL (2005). Gaining perspective on the allergenicity assessment of genetically modified food crops. Expert Rev Clin Immunol 1(4):561-578.

Goodman RE, Hefle SL, Taylor SL, van Ree R (2005). Assessing genetically modified crops to minimize the risk of increased food allergy: a review. Int Arch Allergy Immunol 137(2):153-166.

Goodman RE, Vieths S, Sampson HA, Hill D, Ebisawa M, Taylor SL, van Ree R (2008). Allergenicity assessment of genetically modified crops--what makes sense? Nat Biotechnol 26(1):73-81.

Hoffman M, Bash E, Berger SA, Burke M, Yust I (1992). Fatal necrotizing esophagitis due to *Penicillium chrysogenum* in a patient with acquired immunodeficiency syndrome. Eur J Clin Microbiol Infect Dis 11(12):1158-1160.

Houbraken J, Frisvad JC, Samson RA (2011). Fleming's penicillin producing strain is not *Penicillium chrysogenum* but *P. rubens*. IMA Fungus 2(1):87-95.

Houbraken J, Frisvad JC, Seifert KA, Overy DP, Tuthill DM, Valdez JG *et al.* (2012). New penicillin-producing *Penicillium* species and an overview of section *Chrysogena*. Persoonia 29:78-100.

IFBC (1990). Chapter 4: Safety evaluation of foods and food ingredients derived from microorganisms (International Food Biotechnology Council - IFBC). Regul Toxicol Pharmacol 12(3, Part 2):S114-S128.

JECFA (2006a). General specifications and considerations for enzyme preparations used in food processing. 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 65th JECFA Meetings, 1965-2005. (FAO JECFA Monographs 1). Rome, Italy: Food and Agriculture Organization of the United Nations (FAO), Joint FAO/WHO Expert Committee on Food Additives (JECFA), pp. xxi-xxv. Available at: ftp://ftp.fao.org/docrep/fao/009/a0691e/a0691e.pdf.

JECFA (2006b). Antibacterial activity. 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 65th JECFA Meetings, 1965-2005. (FAO JECFA Monographs 1). Rome, Italy: Food and Agriculture Organization of the United Nations (FAO), Joint FAO/WHO Expert Committee on Food Additives (JECFA), pp. 122-123. Available at: ftp://ftp.fao.org/docrep/fao/009/a0691e/a0691e.pdf.

Kantarcioglu AS, Apaydin H, Yücel A, de Hoog GS, Samson RA, Vural M *et al.* (2004). Central nervous system infection due to *Penicillium chrysogenum*. Mycoses 47(5&6):242-248.

Keung Y-K, Kimbrough R III, Yuen K-Y, Wong W-C, Cobos E (1997). *Penicillium chrysogenum* infection in a cotton farmer with acute myeloid leukemia. Infect Dis Clin Pract 6(7):482-483.

Konishi T, Aoshima T, Mizuhashi F, Choi SSH, Roberts AS (2013). Safety evaluation of glucose oxidase from *Penicillium chrysogenum*. Regul Toxicol Pharmacol 66(1):13-23 & [Corrigendum 66(3):300].

López-Martínez R, Neumann L, González-Mendoza A (1999). Case report: cutaneous penicilliosis due to *Penicillium chrysogenum*. Mycoses 42(4):347-349.

Lyratzopoulos G, Ellis M, Nerringer R, Denning DW (2002). Invasive infection due to *Penicillium* species other than *P. marneffei*. J Infect 45(3):184-195.

MHLW (2000). *Japan's Specifications and Standards for Food Additives, 7th edition*. Tokyo, Japan: Ministry of Health, Labor and Welfare, Japan (MHLW) and Translated by Japan Food Chemical Research Foundation. Available at: http://www.ffcr.or.jp/zaidan/FFCRHOME.nsf/pages/spec.stand.fa [2000, Last updated: 2006/11/30].

MHLW (2011). *List of Existing Food Additives*. Tokyo, Japan: Ministry of Health, Labor and Welfare, Japan (MHLW) and Translated by Japan Food Chemical Research Foundation. Available at: http://www.ffcr.or.jp/zaidan/FFCRHOME.nsf/pages/list-exst.add [Compiled: April 16, 1996, Effective: May 6, 2011].

Mogensen G, Salminen S, O'Brien J, Ouwehand A, Holzapfel W, Shortt C *et al.* (2002). Inventory of microorganisms with a documented history of use in food. In: *Health Benefits and Safety Evaluation of Certain Food Components*. (Bulletin of the International Dairy Federation, No. 377). Brussels, Belgium: International Dairy Federation (IDF), pp. 10-19.

Moss MO (2002). Mycotoxin review – 1. *Aspergillus* and *Penicillium*. Mycologist 16(3):116-119.

Murphy PA, Hendrich S, Landgren C, Bryant CM (2006). Food mycotoxins: an update. J Food Sci 71(5):R51-R65.

O'Brien M, Nielsen KF, O'Kiely P, Forristal PD, Fuller HT, Frisvad JC (2006). Mycotoxins and other secondary metabolites produced in vitro by *Penicillium paneum* Frisvad and *Penicillium roqueforti* Thom isolated from baled grass silage in Ireland. J Agric Food Chem 54(24):9268-9276.

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

OECD (1997b). *In vitro* mammalian chromosome aberration test. In: *OECD Guidelines for the Testing of Chemicals*. (OECD Guideline no 473) [Updated & Adopted 21st July 1997]. Paris, France: Organisation for Economic Co-operation and Development (OECD). Available at: http://www.oecd-ilibrary.org/environment/test-no-473-in-vitro-mammalian-chromosome-aberration-test_9789264071261-en.

OECD (1997c). Mammalian erythrocyte micronucleus test. In: *OECD Guidelines for the Testing of Chemicals*. (OECD Guideline no 474) [Updated & Adopted 21st July 1997]. Paris, France: Organisation for Economic Co-operation and Development (OECD). Available at: http://www.oecd-ilibrary.org/environment/test-no-474-mammalian-erythrocyte-micronucleus-test_9789264071285-en;jsessionid=60s3nuot5c3s1.x-oecd-live-01.

OECD (1998a). *OECD Principles of Good Laboratory Practice*. (Series on Principles of Good Laboratory Practice and Compliance Monitoring, no. 1 [ENV/MC/CHEM(98)17]). Paris, France: Organisation for Economic Co-Operation & Development (OECD), Environment Directorate, Chemicals Group and Management Committee, OECD Environmental Health and Safety Publications. Available at: http://www.oecd.org/document/63/0,3746,en_2649_34381_2346175_1_1_1_1,00.html [As revised in 1997].

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]. Paris, France: Organisation for Economic Co-operation and Development (OECD). Available at: http://www.oecd-ilibrary.org/environment/test-no-408-repeated-dose-90-day-oral-toxicity-study-in-rodents_9789264070707-en;jsessionid=1m6bvwspup322.delta.

Pariza MW, Foster EM (1983). Determining the safety of enzymes used in food processing. J Food Prot 46(5):453-468.

Pariza MW, Johnson EA (2001). Evaluating the safety of microbial enzyme preparations used in food processing: update for a new country. Regul Toxicol Pharmacol 33(2):173-186.

Prasad S, Nema HV (1982). Mycotic infections of cornea. (Drug sensitivity study). Indian J Ophthalmol 30(2):81-85.

Rank C, Nielsen KF, Larsen TO, Varga J, Samson RA, Frisvad JC (2011). Distribution of sterigmatocystin in filamentous fungi. Fungal Biol 115(4&5):406-420.

Rasiah IA, Sutton KH, Low FL, Lin HM, Gerrard JA (2005). Crosslinking of wheat dough proteins by glucoseoxidase and the resulting effects on bread and croissants. Food Chem 89(3):325-332.

Sunesen LO, Stahnke LH (2003). Mould starter cultures for dry sausages-selection, application and effects. Meat Sci 65(3):935-948.

Swoboda-Kopec E, Wroblewska MM, Rokosz A, Luczak M (2003). Mixed bloodstream infection with *Staphylococcus aureus* and *Penicillium chrysogenum* in an immunocompromised patient: case report and review of the literature. Clin Microbiol Infect 9(11):1116-1117.

U.S. FDA (2001). *Bacteriological Analytical Manual Online (BAM)*. Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN). Available at: http://www.911emg.com/Ref%20Library%20ERG/FDA%20Bacteriological%20Analysis.pdf [Page Last Updated: 03/11/2013].

U.S. FDA (2002a). *Agency Response Letter GRAS Notice No. GRN 000089 [Five enzyme preparations from Aspergillus niger: Carbohydrase enzyme preparation, catalase enzyme preparation, glucose oxidase enzyme preparation, pectinase enzyme preparation, and protease enzyme preparation]* (Additional Correspondence in 2004). Submitted by Washington (DC): Enzyme Technical Association to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=89.

U.S. FDA (2002b). *Agency Response Letter GRAS Notice No. GRN000106 [Glucose oxidase enzyme preparation from Aspergillus oryzae carrying a gene encoding a glucose oxidase from Aspergillus niger]*. Submitted by Franklinton (NC): Novozymes North America, Inc. to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=106.

U.S. FDA (2004). *Food Allergen Labeling and Consumer Protection Act of 2004 (Public Law 108-282, Title II)*. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN). Available at: http://www.fda.gov/food/labelingnutrition/FoodAllergensLabeling/GuidanceComplianceRegulatoryInformation/ucm106187.htm.

U.S. FDA (2010). *Guidance for Industry: Enzyme Preparations: Recommendations for Submission of Chemical and Technological Data for Food Additive Petitions and GRAS Notices*. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety Division of Biotechnology and GRAS Notice Review, HFS-255. Available at: http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/IngredientsAdditivesGRASPackaging/ucm217685.htm [Page Last Updated: 05/04/2011].

U.S. FDA (2012). Federal Food, Drug, and Cosmetic Act (FD&C Act): Chapter II: Definitions: Sec. 201. [21 USC §321]: Definitions; generally. In: *U.S. Code—Title 21—Food and Drug, Chapter 9*. Rockville (MD): U.S. Food and Drug Administration (U.S. FDA). Available at: http://www.fda.gov/RegulatoryInformation/Legislation/FederalFoodDrugandCosmeticActFDCAct/FDCActChaptersIandIIShortTitleandDefinitions/default.htm [Page Last Updated: 01/19/2012].

U.S. FDA (2013). *U.S. Code of Federal Regulations (CFR). Title 21—Food and Drugs (Food and Drug Administration)*. Washington (DC): U.S. Government Printing Office (GPO). Available at: http://www.gpo.gov/fdsys/browse/collectionCfr.action?collectionCode=CFR [See Table for CFR sections].

CFR Sections Referenced (Title 21—Food and Drugs)		
Part	**Section §**	**Section Title**
160—Eggs and egg products	160.105	Dried eggs
	160.145	Dried egg whites
	160.185	Dried egg yolks
170—Food additives	170.30	Eligibility for classification as generally recognized as safe (GRAS)
184—Direct food substances affirmed as generally recognized as safe	184.1444	Maltodextrin

USDA (2000). *1994-1996, 1998 Continuing Survey of Food Intakes by Individuals (CSFII) and Diet and Health Knowledge Survey (DHKS)*. [On CD-ROM, PB2000-500027]. Riverdale (MD): U.S. Department of Agriculture (USDA).

USDA (2012). *What We Eat in America: National Health and Nutrition Examination Survey (NHANES): 2009-2010*. Riverdale (MD): U.S. Department of Agriculture (USDA). Available at: http://www.ars.usda.gov/Services/docs.htm?docid=13793#release [Last Modified: 07/16/2012].

Vemulapalli V, Hoseney RC (1998). Glucose oxidase effects on gluten and water soluble. Cereal Chem 75(6):859-862.

Vemulapalli V, Miller KA, Hoseney RC (1998). Glucose oxidase in breadmaking systems. Cereal Chem 75(4):439-442.

Wong CM, Wong KH, Chen XD (2008). Glucose oxidase: natural occurrence, function, properties and industrial applications. Appl Microbiol Biotechnol 78(6):927-938.

Yassin A, Maher A, Moawad MK (1978). Otomycosis: a survey in the eastern province of Saudi Arabia. J Laryngol Otol 92(10):869-876.

APPENDIX A:

Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of the Proposed Food Uses of Sumizyme PGO, a Glucose Oxidase Enzyme Preparation from *Penicillium chrysogenum* PGO 19-162

Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of the Proposed Food Uses of Sumizyme PGO, a Glucose Oxidase Enzyme Preparation from *Penicillium chrysogenum* PGO 19-162

September 13, 2013

INTRODUCTION

Shin Nihon Chemical Co., Ltd. (Shin Nihon) convened a panel (the "Expert Panel") of independent scientists, qualified by their scientific training and relevant national and international experience to evaluate the safety of food ingredients and processing aids, to conduct a critical and comprehensive evaluation of the available pertinent data and information on a glucose oxidase enzyme preparation (containing either 2,000 or 15,000 U glucose activity/g) derived from a strain of *Penicillium chrysogenum* designated as strain PGO 19-162, referred to as Sumizyme PGO hereafter, and to determine whether the proposed uses of Sumizyme PGO in food production would be Generally Recognized as Safe (GRAS) based on scientific procedures. The Expert Panel consisted of the below-signed qualified scientific experts: Dr. Joseph F. Borzelleca (Virginia Commonwealth University School of Medicine), Dr. Michael W. Pariza (Michael W. Pariza Consulting LLC), and Dr. Stanley M. Tarka, Jr. (The Tarka Group, Inc.).

The Expert Panel, independently and collectively, critically evaluated a comprehensive package of scientific information and data compiled from the literature. This information was presented in a dossier provided by Shin Nihon [Documentation Supporting the Evaluation of Sumizyme PGO, a Glucose Oxidase Enzyme Preparation from *Penicillium chrysogenum* PGO 19-162, as Generally Recognized as Safe (GRAS) for Use as a Processing Aid in Food Production], which included an evaluation of all available scientific data and information, both favorable and unfavorable, relevant to the safety of the intended food uses of Shin Nihon's enzyme preparation. This information was prepared in part from a comprehensive search of the scientific literature and also included information characterizing the manufacture of the enzyme preparation, product specifications, supporting analytical data, intended conditions of use, and estimated exposure under the intended uses. In addition, the Expert Panel evaluated other information deemed appropriate or necessary.

Following its independent critical evaluation, the Expert Panel unanimously concluded that the intended uses described herein of Shin Nihon's Sumizyme PGO, meeting appropriate food-grade specifications, and manufactured consistent with current Good Manufacturing Practice (cGMP), are GRAS based on scientific procedures. A summary of the basis for the Expert Panel's conclusion appears below.

SUMMARY AND BASIS FOR GRAS

Shin Nihon intends to market Sumizyme PGO, a glucose oxidase enzyme preparation from a strain of *Penicillium chrysogenum* designated as strain PGO 19-162, as a processing aid in the production of foods and beverages in the United States (U.S.). Sumizyme PGO is formulated to a specific activity of either 2,000 or 15,000 U glucose oxidase activity/g. The 2,000 U/g enzyme preparation is designated as Sumizyme PGO 2000 and the 15,000 U/g enzyme preparation is designated as Sumizyme PGO 15000. The two enzyme preparations are collectively referred to as Sumizyme PGO herein.

Glucose oxidase is a well-characterized enzyme that catalyzes the oxidation of β-D-glucose to D-glucono-1,5-lactone (D-glucono-δ-lactone) in the presence of molecular oxygen, releasing H_2O_2 in the process. D-glucono-1,5-lactone is subsequently hydrolyzed to gluconic acid by non-enzymatic means. The enzyme has a long history of use in the food and beverage industry with current applications including use as a preservative and stabilizer. Specifically, the enzyme is often applied (along with catalase) to foods to prevent non-enzymatic browning through the removal of residual amounts of glucose and to foods and beverages to prevent color and flavor loss through the removal of residual oxygen (as reviewed by Crueger and Crueger, 1990; Wong *et al*., 2008 and Bankar *et al*., 2009). Glucose oxidase also is used to strengthen gluten contained in wheat flour-based starch products, such as breads and other baked products, thereby improving quality (Vemulapalli and Hoseney, 1998; Vemulapalli *et al.*, 1998; Rasiah *et al.*, 2005). It is the Expert Panel's understanding that Sumizyme PGO is intended as an alternative to current glucose oxidase preparations applied in the food and beverage industry at the minimum levels necessary to achieve the intended effect (cGMP). The intended uses of Sumizyme PGO as a processing aid are therefore consistent with the current applications of glucose oxidase in foods and beverages, which include those currently authorized for use by the U.S. Food and Drug Administration (FDA) (21CFR §160.105, 160.145, 160.185) (U.S. FDA, 2013) or considered to be GRAS through the self-GRAS determination procedure with no objections from FDA (GRAS Notice No. 000089, U.S. FDA, 2002a; GRAS Notice No. 000106, U.S. FDA, 2002b). As Sumizyme PGO is intended as an alternative source of glucose oxidase replacing currently approved and used glucose oxidase preparations, the use of Sumizyme PGO should not significantly increase current dietary intakes of glucose oxidase.

The strain of *P. chrysogenum* (PGO 19-162) used for the production of glucose oxidase was originally isolated from a soil sample, and was selected as the production strain based on its capacity to produce high levels of glucose oxidase activity. Strain PGO 19-162 has been deposited in a recognized culture collection and has been identified by 2 independent laboratories as belonging to the species *P. chrysogenum* by morphological and phylogenetic means, respectively. The secondary metabolite profile analytically determined for the strain also is consistent with the species *P. chrysogenum*. The Expert Panel concluded that the

production organism was sufficiently characterized for use in the safety assessment of the enzyme.

Information detailing the fermentation and purification procedures used for production of Sumizyme PGO were presented to the Expert Panel. The enzyme preparation is manufactured in compliance with cGMP and the principles of Hazard Analysis and Critical Control Points (HACCP). Appropriate control measures and analytical testing are implemented during manufacturing to ensure that transfer of viable organisms, including the production strain, to the final enzyme preparations does not occur. The enzyme is produced through quality-controlled fermentation and purification processes using food-grade raw materials and processing aids and permitted filter aids. The fermentation process involves cultivation of a seed inoculum followed by a main culture cultivated under submerged fermentation. The purification process consists of a series of filtration steps, including filtration through a filter press to remove the production strain and ultrafiltration (also concentration) to remove proteins, saccharides, lipids, salts, and other compounds less than 5,000 MW. The enzyme is secreted from the production strain into the fermentation medium and therefore occurs in the concentrated solution, which is subjected to microfiltration to remove potential contaminating microorganisms and residual amounts of the production strain. Following purification, the enzyme is precipitated and washed with ethanol and then isolated by centrifugation. The ethanol-containing supernatant (consisting of 92% ethanol) is removed and discarded. The solid precipitate containing the isolated enzyme is then dried by vacuum. Alternatively, the filtered enzyme solution is dried by a spray drying process. The solid product is sifted, resulting in a final powdered enzyme product that possesses a glucose oxidase activity of 60,000 U/g. The glucose oxidase activity of the powdered product is adjusted/standardized with the addition of food-grade maltodextrin to produce Sumizyme PGO formulations with a specific activity of either 2,000 or 15,000 U glucose oxidase activity/g (Sumizyme PGO 2000 and Sumizyme PGO 15000, respectively). Maltodextrin is affirmed as GRAS in the U.S. for use in foods with no limitation other than cGMP (21CFR §184.1444) (U.S. FDA, 2013). The maltodextrin used in the formulation of Sumizyme PGO is food-grade. Therefore, there are no safety concerns with the use of maltodextrin for standardization purposes. No other diluents, stabilizers, or preservatives are added to the enzyme. The composition of the final enzyme preparations is presented in Table 1.

Table 1 Composition of Sumizyme PGO Enzyme Preparations	
Parameter	**Content**
Sumizyme PG 2000	
Glucose oxidase specific activity	Not less than 2,000 U/g
Enzyme solids (total organic solids)	2.0 to 3.0%
Water	0.06
Ash	0.44
Maltodextrin	97 to 97.5%
Sumizyme PG 15000	
Glucose oxidase specific activity	Not less than 15,000 U/g
Enzyme solids (total organic solids)	15 to 25%
Water	0.5
Ash	3.5
Maltodextrin	77 to 82 %

Appropriate food-grade product specifications have been established for Sumizyme PGO 2000 and Sumizyme PGO 15000 and are presented in Table 2.

Table 2 Specifications for Sumizyme PGO 2000 and Sumizyme PGO 15000	
Specification Parameter	**Specification Requirements**
Assay (Activity)	Sumizyme PGO 2000: NLT 2,000 U/g
	Sumizyme PGO 15000: NLT 15,000 U/g
Enzyme solids (total organic solids)	Sumizyme PGO 2000: 2.0 to 3.0%
	Sumizyme PGO 15000: 15 to 25%
Arsenic	NMT 3 mg/kg
Lead	NMT 1 mg/kg
Total aerobic plate count	NMT 50,000 CFU/g
Coliforms	NMT 30 CFU/g
Escherichia coli	Negative in 25 g
Salmonella species	Negative in 25 g
Mold	NMT 100 CFU/g
Antibacterial activity	Negative

CFU = colony forming units; NLT = not less than; NMT = not more than

The specifications for both Sumizyme PGO 2000 and Sumizyme PGO 15000 are identical apart from glucose oxidase activity and total organic solids content (TOS). The Expert Panel confirms that the specifications comply with the current purity and microbial limits established for enzyme preparations in the Food Chemicals Codex (FCC, 2012) and by the Joint FAO/WHO Committee on Food Additives (JECFA) (JECFA, 2006). The product specifications also include specifications for glucose oxidase activity, antibacterial activity, and mold. All analyses are nationally or internationally recognized methods or are Shin Nihon validated methods. The Expert Panel reviewed the results from 3 non-consecutive commercial lots of each enzyme

preparation and confirm that the data demonstrate that the manufacturing process produces a consistent material in conformance with the product specifications. The Expert Panel also reviewed solvent analyses from 3 non-consecutive lots of the concentrated enzyme preparation prior to dilution with maltodextrin (enzyme concentrate containing 60,000 U glucose activity/g), which demonstrate that residual ethanol is absent from the concentrate prior to formulation as assessed by gas chromatography (*i.e.*, below the limit of detection of 0.1 g/100 g). The results indicate a lack of any significant residual ethanol levels in the final enzyme preparations. The final enzyme preparations do not contain secondary enzyme activities as established through analytical testing of 3 non-consecutive lots of each of Sumizyme PGO 2000 and Sumizyme PGO 15000.

The enzyme preparations are stored in fiber drums each lined with a polyethylene bag and sealed with a ring seal. The product is stored in sealed containers under cool (approximately 20°C), dry conditions. The Expert Panel reviewed the analytical results of a stability study on the Sumizyme PGO 15000 bulk material and concluded that glucose oxidase activity is stable for at least 6 months under these storage conditions. The enzyme is thermally unstable at temperatures in excess of 50°C and is stated to be completely inactivated by heating at a temperature of 80°C for 15 minutes.

Sumizyme PGO is proposed for use in the production of foods from selected food categories, including alcoholic beverages; baked goods and baking mixes; beverages and beverage bases; coffee and tea; condiments and relishes; egg products; gelatins, puddings, and fillings; grain products and pastas; processed fruits and fruit juices; processed vegetables and vegetable juices; and snack foods. The maximum level of use of Sumizyme PGO expressed on a TOS basis is 0.003% per kg in foods and 0.0003% per kg in beverages. The enzyme preparation is added directly to food, but the glucose oxidase enzyme is inactivated at a temperature of 80°C (thermal instability is achieved at temperatures greater than 50°C), and therefore, would be inactivated under food processing conditions consisting of high temperatures, such as occurs during baking/cooking and pasteurization/sterilization. In accordance with the FDA guidance for industry on enzyme preparations that are the subject of GRAS notices, an estimate of the dietary exposure to the enzyme preparation was performed on a TOS basis. The maximum use level expressed as TOS was used in the assessment on the basis that, although rendered inactive through food processing, Sumizyme PGO will not be removed from the proposed foods and beverages. From the assessment, the estimated 90th percentile daily intake of TOS from the use of Sumizyme PGO in the total U.S. population was calculated to be 0.27 mg/kg body weight/day. The greatest estimated daily intakes calculated among all population groups on a body weight basis occurred in infants and children. Estimated 90th percentile daily intakes in these population groups were calculated to be 0.64 and 0.48 mg/kg body weight/day, respectively.

The Expert Panel critically evaluated the data and information characterizing the safety of Sumizyme PGO, including safety of the production strain (toxigenicity, pathogenicity, and

antibiotic production) and allergenicity. The safety of Sumizyme PGO for use in foods was assessed according to the guidelines developed by Pariza and Foster (1983), Pariza and Johnson (2001), and the International Food Biotechnology Council (IFBC) (IFBC, 1990) on assessing the safety of microbial enzyme preparations used in foods.

The primary consideration in evaluating enzyme safety is the safety of the production strain for which the primary concern is its toxigenic potential. *P. chrysogenum* is a filamentous fungus with a history of safe use as a starter culture for the production of dry sausages (Sunesen and Stahnke, 2003) and in the fermentation and biopreservation of meat (Mogensen *et al.*, 2002). *P. chrysogenum* also is a current source of a commercialized glucose oxidase enzyme preparation used in food processing (Amfep, 2009), and *Penicillium* (species not specified) also is an approved source of glucose oxidase in Japan (MHLW, 2011). However, considering that filamentous fungi, including *P. chrysogenum*, are well-known producers of antibiotics, mycotoxins, and other secondary metabolites and that the production of these substances may vary from strain to strain, an assessment of the safety of *P. chrysogenum* PGO 19-162, with emphasis on the toxicogenic potential and antibiotic production of the strain was conducted.

For filamentous fungi, such as *P. chrysogenum*, the oral toxins of concern are mycotoxins (small molecular weight organic molecules, usually less than 1,000 Da in size) (Pariza and Johnson, 2001). Therefore, the Expert Panel evaluated the data on the secondary metabolites produced by the production strain *P. chrysogenum* PGO 19-162 and the potential presence of these metabolites in the final enzyme preparations. It is noted that although *P. chrysogenum* does not produce the well-known mycotoxins of typical concern to food safety, the species is known to produce the mycotoxins roquefortine C, PR toxin, and secalonic acids, as well as the secondary metabolite chrysogine (Frisvad *et al.*, 2004). Therefore, Shin Nihon commissioned secondary metabolite analysis of both the production strain and the concentrated enzyme preparation (containing 60,000 U glucose activity/g). Analyses of the production strain *P. chrysogenum* PGO 19-162 was shown to produce the mycotoxin roquefortine C and the secondary metabolite chrysogine (but not PR toxin or secalonic acids), consistent with the secondary metabolite profile of the species *P. chrysogenum* reported in the scientific literature (Frisvad *et al*., 2004). Mycotoxin analysis of the concentrated enzyme preparation demonstrate absence of the mycotoxin roquefortine C in the preparation. The analytical results on the concentrated enzyme preparation further demonstrate absence of the major food-contaminating mycotoxins, including ochratoxin A, aflatoxin (B_1, B_2, G_1, G_2), zearalenone, sterigmatocystin, and T-2 toxin, although the latter four are known not to be produced by *Penicillium* species (Bennett and Klich, 2003; Frisvad *et al.*, 2004; Rank *et al.,* 2011). Although standards for the secondary metabolite chrysogine are not readily available for analytical testing, the mycotoxin results for Sumizyme PGO indicate that the purification steps applied in the manufacture of Sumizyme PGO are sufficient in removing any toxins of concern. Sumizyme PGO is thus considered to be free of toxic metabolites.

P. chrysogenum is a well-known producer of β-lactam antibiotics, including meleagrin, xanthocillin, sorrentanone, and sorbicillin (Frisvad *et al.*, 2004). The Expert Panel reviewed analytical data on the production strain which indicated that *P. chrysogenum* PGO 19-162 produces detectable levels of the antibiotics meleagrin and xanthocillin X. Analyses demonstrate that the production strain does not produce any detectable levels of antibacterial activity and that the final enzyme preparations (Sumizyme PGO 2000 and Sumizyme PGO 15000) also lack antibacterial activity.

The pathogenicity of the production strain also was considered. A critical evaluation of the available scientific literature indicates that strains of *P. chrysogenum* are generally considered to be non-pathogenic under normal circumstances, with infections being extremely rare and limited to opportunistic infections (Upshaw, 1974; Yassin *et al.*, 1978; Eschete *et al.*, 1981; Prasad and Nema, 1982; Hoffman *et al.*, 1992; D'Antonio *et al*., 1997; Keung *et al.,* 1997; López-Martínez *et al.*, 1999; Botterel *et al.*, 2002; Lyratzopoulos *et al.*, 2002; Swoboda-Kopec *et al.*, 2003; Kantarcioğlu *et al.*, 2004; Barcus *et al.,* 2005). Appropriate control measures are implemented during the manufacture of Sumizyme PGO to ensure that transfer of viable organisms to the final enzyme preparations does not occur.

The Expert Panel concluded that there was sufficient generally available published information to conclude that the production strain *P. chrysogenum* PGO 19-162 is non-pathogenic and non-toxicogenic and therefore are safe and suitable for use in the production of enzyme preparations intended for use in food applications.

The Expert Panel critically evaluated the toxicological data on Sumizyme PGO, consisting of a battery of classical toxicological tests, including a repeat dose 90-day oral toxicity study conducted in rats and a series of genotoxicity tests (Konishi *et al*., 2013). All tests were performed in compliance with the Organisation of Economic Co-operation and Development (OECD) Principles of Good Laboratory Practice (GLP) and internationally accepted guidelines for the toxicity testing of chemicals where applicable (*i.e*., OECD test guideline 408, 471, 473, and 474).

The 90-day toxicity study was conducted in Crl:CD(SD) rats administered Sumizyme PGO by gavage at doses of 93, 19.3, or 193 mg TOS/kg body weight/day. Based on the results of this study, the no-observed-adverse-effect level (NOAEL) for Sumizyme PGO was determined to be 193 mg TOS/kg body weight/day, the highest dose tested. Sumizyme PGO also was determined to be non-genotoxic in a series of *in vitro* and *in vivo* genotoxicity tests (bacterial reverse mutation assay, *in vitro* chromosomal aberration test in human lymphocytes, and an *in vivo* combined erythrocyte micronucleus/comet assay in rats). That Sumizyme PGO is free of oral toxins is supported by these data. The Expert Panel evaluated the Sumizyme PGO enzyme preparation using the Pariza-Johnson decision tree and concluded that it is safe and acceptable for use in food (see Attachment A).

To address the issue of allergenicity, the Expert Panel reviewed the results of a sequence homology search conducted using the web-based AllergenOnline database (version 13) to determine whether the glucose oxidase produced by *P. chrysogenum* PGO 19-162 contains amino acid sequences similar to known allergens that might produce an allergenic response. A high degree of similarity (E value[1] = 2.7 e-021) was identified between the full length *P. chrysogenum* PGO 19-162 glucose oxidase amino acid sequence and the Mala s 12 allergen precursor produced by the fungal species *Malassezia sympodialis*. The percent identity[2] of the amino acid sequences however was low at 31%, indicating a low potential for cross-reactivity to this allergen. A second homology search was conducted according to the 80 amino acid sliding window alignment approach outlined by the FAO/WHO (2001) and the Codex Alimentarius Commission (2003a,b) for evaluating newly expressed proteins produced by recombinant-DNA microorganisms and by genetically modified crops. Using this search strategy, one identity match of 45%[3] to the Mala s 12 allergen precursor produced by *Malassezia sympodialis* was identified, consistent with the results of the full-length alignment search. However, Goodman *et al.* (2005) and Goodman and Hefle (2005) discuss this approach of evaluating segments of 80 amino acids to be conservative and precautionary. Therefore, considering that the percent identity of the full length amino acid sequences was low at 31%, only a low potential for cross-reactivity to the Mala s 12 allergen precursor exists. No exact matches were identified when searching for 8 contiguous amino acids. It is further noted that no matches to any of the 8 major food allergens were identified. The 8 major food allergens are milk, egg, peanuts, tree nuts, soybeans, wheat, fish, and Crustacean shellfish as stipulated under the Food Allergen Labeling and Consumer Protection Act of 2004 (U.S. FDA, 2004). Based on the data reviewed, no evidence exists that might indicate that Sumizyme PGO would produce an allergenic response following consumption of foods to which the enzyme has been added. Moreover, there is no evidence from the available scientific literature indicating allergenicity to glucose oxidase in consumers of foods to which the enzyme has been added. The current history of safe use of glucose oxidase in foods and beverages corroborates the conclusion that glucose oxidase preparations are of low allergenic potential. Furthermore, the enzyme is not anticipated to result in allergenicity in consumers on the basis that the enzyme would be inactivated and denatured under the conditions of food processing. The Expert Panel therefore concluded that the allergenicity of the enzyme preparation is not a health concern.

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[1] The *E*-value (expectation value) is a calculated value that indicates the degree of similarity between protein sequences, where a very low *E*-value (*e.g.*, 10e-30) indicates a high degree of similarity, while a value of 1 or higher indicates the proteins are not likely to be related in evolution or structure.

[2] Proteins with greater than 70% identical primary amino acid sequences throughout the length of the protein compared to an allergen are commonly cross-reactive, while those with less than 50% identity are rarely cross-reactive (Aalberse, 2000).

[3] According to the approach adopted by the Codex Alimentarius Commission, significant homology is defined as an identity match of greater than 35%, and in such instances, cross-reactivity with the known allergen should be considered a possibility. The 35% identity for 80 amino acid segments is a suggested guideline proposed by the Codex Alimentarius Commission for evaluating newly expressed proteins produced by recombinant-DNA microorganisms (Codex Alimentarius Commission, 2003a), and is the same approach proposed for genetically modified crops (Codex Alimentarius Commission, 2003b).

CONCLUSION

We, the members of the Expert Panel, have independently and collectively critically evaluated the information summarized above and conclude that the intended uses as a processing aid in the production of food of Shin Nihon's (Shin Nihon Chemical Co., Ltd.) glucose oxidase enzyme preparation (Sumizyme PGO) produced by fermentation from *Penicillium chrysogenum* strain PGO 19-162, manufactured in accordance with current Good Manufacturing Practice and meeting appropriate food-grade specifications, are safe.

We, the members of the Expert Panel, have independently and collectively critically evaluated the information summarized above and conclude that the intended uses as a processing aid in the production of food of Shin Nihon's (Shin Nihon Chemical Co., Ltd.) glucose oxidase enzyme preparation (Sumizyme PGO) produced by fermentation from *Penicillium chrysogenum* strain PGO 19-162, manufactured in accordance with current Good Manufacturing Practice and meeting appropriate food-grade specifications, are Generally Recognized as Safe (GRAS) based on scientific procedures.

It is our opinion that other qualified experts would concur with these conclusions.

(b) (6)

12 November 2013

Prof. Joseph F. Borzelleca Ph.D.
Virginia Commonwealth University School of Medicine

Date

(b) (6)

13 November 2013

Prof. Michael W. Pariza Ph.D.
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Date

(b) (6)

14 November 2013

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Date

REFERENCES

Aalberse RC (2000). Structural biology of allergens. J Allergy Clin Immunol 106(2):228-238. Cited In: Goodman and Wise, 2006.

Amfep (2009). *List of Enzymes October 2009*. Brussels, Belgium: Association of Manufacturers and Formulators of Enzyme Products (Amfep). Available at: http://amfep.drupalgardens.com/sites/amfep.drupalgardens.com/files/Amfep-List-of-Commercial-Enzymes.pdf.

Bankar SB, Bule MV, Singhal RS, Ananthanarayan L (2009). Glucose oxidase--an overview. Biotechnol Adv 27(4):489-501.

Barcus AL, Burdette SD, Herchline TE (2005). Intestinal invasion and disseminated disease associated with *Penicillium chrysogenum*. Ann Clin Microbiol Antimicrob 4:21.

Bennett JW, Klich M (2003). Mycotoxins. Clin Microbiol Rev 16(3):497-516.

Botterel F, Cordonnier C, Barbier V, Wingerstmann L, Liance M, Coste A *et al.* (2002). Aspergillus fumigatus causes in vitro electrophysiological and morphological modifications in human nasal epithelial cells. Histol Histopathol 17(4):1095-1101.

Codex Alimentarius Commission (2003a). *Report of the Fourth Session of the Codex Ad hoc Intergovernmental Task Force on Foods Derived from Biotechnology* (Yokohama, Japan 11-14 March 2003). (ALINORM 03/34A). Twenty-sixth Session, June 30 - July 7 2003. Rome, Italy: Food and Agriculture Organization of the United Nations (FAO), World Health Organization (WHO), Codex Alimentarius Commission (CODEX). Available at: ftp://ftp.fao.org/docrep/fao/meeting/006/y9220e.pdf.

Codex Alimentarius Commission (2003b). *Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant DNA Plants*. (CODEX-STAN CAC/GL 45-2003). Rome, Italy: Codex Alimentarius Commission (CODEX-STAN). Available at: http://www.who.int/foodsafety/biotech/en/codex_guidelines_plants.pdf.

Crueger A, Crueger W (1990). Glucose transforming enzymes. In: Fogarty WM, Kelly CT, editors. *Microbial Enzymes and Biotechnology, 2nd edition*. London, Engl. / New York (NY): Elsevier Applied Science, pp. 177-226.

D'Antonio D, Violante B, Farina C, Sacco R, Angelucci D, Masciulli M *et al.* (1997). Necrotizing pneumonia caused by Penicillium chrysogenum. J Clin Microbiol 35(12):3335-3337.

Eschete ML, King JW, West BC, Oberle A (1981). Penicillium chrysogenum endophthalmitis. Mycopathologia 74(2):125-127.

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. Rome, Italy: Food and Agriculture Organization of the United Nations (FAO), World Health Organization (WHO). Available at: ftp://ftp.fao.org/es/esn/food/allergygm.pdf.

FCC (2012). Enzyme preparations. In: *Food Chemicals Codex, 8th edition*. Rockville (MD): United States Pharmacopeial Convention (USP), pp. 375-380.

Frisvad JC, Smedsgaard J, Larsen TO, Samson RA (2004). Mycotoxins, drugs and other extrolites produced by species in *Penicillium* subgenus *Penicillium.* Stud Mycol 49:201-241.

Goodman RE, Hefle SL (2005). Gaining perspective on the allergenicity assessment of genetically modified food crops. Expert Rev Clin Immunol 1(4):561-578.

Goodman RE, Wise J (2006). *Bioinformatic Analysis of Proteins in Golden Rice 2 to Assess Potential Allergenic Cross-Reactivity* (Study No. BIO-02-2006). Lincoln (NE): Food Allergy Research and Resource Program, Food Science and Technology, University of Nebraska. Available at: http://www.allergenonline.org/Golden%20Rice%202%20Bioinformatics%20FARRP%202006.pdf.

Goodman RE, Hefle SL, Taylor SL, van Ree R (2005). Assessing genetically modified crops to minimize the risk of increased food allergy: a review. Int Arch Allergy Immunol 137(2):153-166.

Hoffman M, Bash E, Berger SA, Burke M, Yust I (1992). Fatal necrotizing esophagitis due to *Penicillium chrysogenum* in a patient with acquired immunodeficiency syndrome. Eur J Clin Microbiol Infect Dis 11(12):1158-1160.

IFBC (1990). Chapter 4: Safety evaluation of foods and food ingredients derived from microorganisms (International Food Biotechnology Council - IFBC). Regul Toxicol Pharmacol 12(3, Part 2):S114-S128.

JECFA (2006). General specifications and considerations for enzyme preparations used in food processing. 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 65th JECFA Meetings, 1965-2005. (FAO JECFA Monographs 1). Rome, Italy: Food and Agriculture Organization of the United Nations (FAO), Joint FAO/WHO Expert Committee on Food Additives (JECFA), pp. xxi-xxv. Available at: ftp://ftp.fao.org/docrep/fao/009/a0691e/a0691e.pdf.

Kantarcioglu AS, Apaydin H, Yücel A, de Hoog GS, Samson RA, Vural M *et al.* (2004). Central nervous system infection due to *Penicillium chrysogenum*. Mycoses 47(5&6):242-248.

Keung Y-K, Kimbrough R III, Yuen K-Y, Wong W-C, Cobos E (1997). *Penicillium chrysogenum* infection in a cotton farmer with acute myeloid leukemia. Infect Dis Clin Pract 6(7):482-483.

Konishi T, Aoshima T, Mizuhashi F, Choi SSH, Roberts AS (2013). Safety evaluation of glucose oxidase from *Penicillium chrysogenum*. Regul Toxicol Pharmacol 66(1):13-23 & [Corrigendum 66(3):300].

López-Martínez R, Neumann L, González-Mendoza A (1999). Case report: cutaneous penicilliosis due to *Penicillium chrysogenum*. Mycoses 42(4):347-349.

Lyratzopoulos G, Ellis M, Nerringer R, Denning DW (2002). Invasive infection due to *Penicillium* species other than *P. marneffei*. J Infect 45(3):184-195.

MHLW (2011). *List of Existing Food Additives*. Tokyo, Japan: Ministry of Health, Labor and Welfare, Japan (MHLW) and Translated by Japan Food Chemical Research Foundation. Available at: http://www.ffcr.or.jp/zaidan/FFCRHOME.nsf/pages/list-exst.add [Compiled: April 16, 1996, Effective: May 6, 2011].

Mogensen G, Salminen S, O'Brien J, Ouwehand A, Holzapfel W, Shortt C *et al.* (2002). Inventory of microorganisms with a documented history of use in food. In: *Health Benefits and Safety Evaluation of Certain Food Components*. (Bulletin of the International Dairy Federation, No. 377). Brussels, Belgium: International Dairy Federation (IDF), pp. 10-19.

Pariza MW, Foster EM (1983). Determining the safety of enzymes used in food processing. J Food Prot 46(5):453-468.

Pariza MW, Johnson EA (2001). Evaluating the safety of microbial enzyme preparations used in food processing: update for a new country. Regul Toxicol Pharmacol 33(2):173-186.

Prasad S, Nema HV (1982). Mycotic infections of cornea. (Drug sensitivity study). Indian J Ophthalmol 30(2):81-85.

Rank C, Nielsen KF, Larsen TO, Varga J, Samson RA, Frisvad JC (2011). Distribution of sterigmatocystin in filamentous fungi. Fungal Biol 115(4&5):406-420.

Rasiah IA, Sutton KH, Low FL, Lin HM, Gerrard JA (2005). Crosslinking of wheat dough proteins by glucoseoxidase and the resulting effects on bread and croissants. Food Chem 89(3):325-332.

Sunesen LO, Stahnke LH (2003). Mould starter cultures for dry sausages-selection, application and effects. Meat Sci 65(3):935-948.

Swoboda-Kopec E, Wroblewska MM, Rokosz A, Luczak M (2003). Mixed bloodstream infection with *Staphylococcus aureus* and *Penicillium chrysogenum* in an immunocompromised patient: case report and review of the literature. Clin Microbiol Infect 9(11):1116-1117.

U.S. FDA (2002a). *Agency Response Letter GRAS Notice No. GRN 000089 [Five enzyme preparations from Aspergillus niger: Carbohydrase enzyme preparation, catalase enzyme preparation, glucose oxidase enzyme preparation, pectinase enzyme preparation, and protease enzyme preparation]* (Additional Correspondence in 2004). Submitted by Washington (DC): Enzyme Technical Association to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=89.

U.S. FDA (2002b). *Agency Response Letter GRAS Notice No. GRN000106 [Glucose oxidase enzyme preparation from Aspergillus oryzae carrying a gene encoding a glucose oxidase from Aspergillus niger]*. Submitted by Franklinton (NC): Novozymes North America, Inc. to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=106.

U.S. FDA (2004). *Food Allergen Labeling and Consumer Protection Act of 2004 (Public Law 108-282, Title II)*. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN). Available at: http://www.fda.gov/food/labelingnutrition/FoodAllergensLabeling/GuidanceComplianceRegulatoryInformation/ucm106187.htm.

U.S. FDA (2013). *U.S. Code of Federal Regulations (CFR). Title 21—Food and Drugs (Food and Drug Administration)*. Washington (DC): U.S. Government Printing Office (GPO). Available at: http://www.gpo.gov/fdsys/browse/collectionCfr.action?collectionCode=CFR [See Table for CFR sections].

CFR Sections Referenced (Title 21—Food and Drugs)		
Part	**Section §**	**Section Title**
160—Eggs and egg products	160.105	Dried eggs
	160.145	Dried egg whites
	160.185	Dried egg yolks
184—Direct food substances affirmed as generally recognized as safe	184.1444	Maltodextrin

Upshaw CB Jr. (1974). Penicillium endocarditis of aortic valve prosthesis. J Thorac Cardiovasc Surg 68(3):428-431.

Vemulapalli V, Hoseney RC (1998). Glucose oxidase effects on gluten and water soluble. Cereal Chem 75:859-862.

Vemulapalli V, Miller KA, Hoseney RC (1998). Glucose oxidase in breadmaking systems. Cereal Chem 75:439-442.

Wong CM, Wong KH, Chen XD (2008). Glucose oxidase: natural occurrence, function, properties and industrial applications. Appl Microbiol Biotechnol 78(6):927-938.

Yassin A, Maher A, Moawad MK (1978). Otomycosis: a survey in the eastern province of Saudi Arabia. J Laryngol Otol 92(10):869-876.

ATTACHMENT A

Pariza-Johnson Decision Tree Analysis

This analysis is based on the Decision Tree of MW Pariza and EA Johnson (2001): *Evaluating the Safety of Microbial Enzyme Preparations Used in Food Processing: Update for a New Century*, Regulatory Toxicology and Pharmacology, 33:173-186. Decision points that do not pertain are included for completeness but crossed out.

1. Is the production strain genetically modified?
   If yes, go to 2. If no, go to 6. **NO**

~~2. Is the production strain modified using rDNA techniques?~~
   ~~If yes, go to 3. If no, go to 5.~~

~~3. Issues relating to the introduced DNA are addressed in 3a–3e.~~

~~3a. Do the expressed enzyme product(s) which are encoded by the introduced DNA have a history of safe use in food?~~
   ~~If yes, go to 3c. If no, go to 3b~~

~~3b. Is the NOAEL for the test article in appropriate short-term oral studies sufficiently high to ensure safety?~~
   ~~If yes, go to 3c. If no, go to 12.~~

~~3c. Is the test article free of transferable antibiotic resistance gene DNA?~~
   ~~If yes, go to 3e. If no, go to 3d.~~

~~3d. Does the resistance gene(s) code for resistance to a drug substance used in treatment of disease agents in man or animal? If yes, go to 12. If no, go to 3e.~~

~~3e. Is all other introduced DNA well characterized and free of attributes that would render it unsafe for constructing microorganisms to be used to produce food-grade products?~~
   ~~If yes, go to 4. If no, go to 12.~~

~~4. Is the introduced DNA randomly integrated into the chromosome?~~
   ~~If yes, go to 5. If no, go to 6.~~

~~5. Is the production strain sufficiently well characterized so that one may reasonably conclude that unintended pleiotropic effects which may result in the synthesis of toxins or other unsafe metabolites will not arise due to the genetic modification method that was employed?~~
   ~~If yes, go to 6. If no, go to 7.~~

6. Is the production strain derived from a safe lineage, as previously demonstrated by repeated assessment via this evaluation procedure?
   If yes, the test article is ACCEPTED. If no, go to 7. **NO**

7. Is the organism nonpathogenic? **YES**
   If yes, go to 8. If no, go to 12.

8. Is the test article free of antibiotics? **YES**
   If yes, go to 9. If no, go to 12.

9. Is the test article free of oral toxins known to be produced by other members of the same species?
   If yes, go to 11. If no, go to 10. **YES**

~~10. Are the amounts of such toxins in the test article below levels of concern?~~
   ~~If yes, go to 11. If no, go to 12.~~

11. Is the NOAEL for the test article in appropriate oral studies sufficiently high to ensure safety?
   If yes, the test article is ACCEPTED. **YES, the test article, Sumizyme PGO, is ACCEPTED**

~~12. An undesirable trait or substance may be present and the test article is not acceptable for food use. If the genetic potential for producing the undesirable trait or substance can be permanently inactivated or deleted, the test article may be passed through the decision tree again.~~

SUBMISSION END