Application for the Authorisation of Glucose Oxidase from *Penicillium chrysogenum* Strain PGO 19-162 as a Food Enzyme in the European Union

Pursuant to

Regulation (EC) No 1332/2008 of the European Parliament and Council of 16 December 2008 on Food Enzyme

2.2 PUBLIC SUMMARY

NON-CONFIDENTIAL

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4 March 2015

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INTRODUCTION

Shin Nihon Chemical Co., Ltd (Shin Nihon) wishes to market glucose oxidase derived from a non-genetically modified strain of *Penicillium chrysogenum*, designated as strain PGO 19-162, as a food enzyme in the European Union (EU). The food enzyme is herein referred to as "the glucose oxidase food enzyme". The application is being made to allow the glucose oxidase food enzyme to be added to the Community list of food enzymes.

TECHNICAL DATA

Identity of the Food Enzyme

The food enzyme subject of this application is glucose oxidase derived from a non-genetically modified strain of *Penicillium chrysogenum*, designated as strain PGO 19-162. The enzyme is identified by the following systematic names and numbers:

Source (Strain):	*Penicillium chrysogenum* strain PGO 19-162
Common Name:	Glucose oxidase
Shin Nihon Enzyme Name/Abbreviation:	PGO
Other names:	Glucose oxyhydrase; glucose aerodehydrogenase; β-D-glucose oxidase; D -glucose oxidase; D-glucose-1-oxidase; glucose oxhydrase; GOX; GOD

Enzyme Classification Number of Enzyme Commission (EC) of the International Union of Biochemistry and Molecular Biology (IUBMB)]:	EC 1.1.3.4
Chemical/Systematic Name:	β- D-glucose:oxygen-1-oxidoreductase
Chemical Abstracts Service (CAS) Number:	9001-37-0
European Inventory of Existing Chemical Substances (EINECS) Number or European List of Notified Chemical Substances (ELINCS) Number:	232-601-0

Chemical Composition and Properties of the Food Enzyme

The glucose oxidase food enzyme produced with *P. chrysogenum* strain PGO 19-162 is manufactured as an ultra-filtered liquid concentrate that does not contain any added diluents and is characterised by its glucose oxidase activity. The enzyme is not modified by a post-translational process or by technological procedures, and is not protein engineered.

The food enzyme is routinely analysed to confirm the purity and absence of any external contaminants. The specifications established for the food enzyme comply with the current purity and microbial limits established for enzyme preparations by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and in the Food Chemicals Codex (FCC).

Glucose oxidase catalyses 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. This enzyme reaction does not require any co-factors. D-glucono-1,5-lactone is subsequently hydrolysed to gluconic acid by non-enzymatic means. Hydrogen peroxide (H_2O_2) also is produced as a by-product of the reaction. The optimum temperature range and pH for the glucose oxidase activity of the food enzyme have been established experimentally. Glucose oxidase activity becomes thermally unstable at high temperatures and is therefore heat-denatured (inactivated) during food processing at high temperatures. The shelf-life of a representative final formulated enzyme preparation has been established as 12 months when stored under the recommended conditions.

Source Materials and Manufacturing Process

Shin Nihon has established appropriate quality control procedures to ensure production of a high quality and safe food enzyme, including use of a safe production strain. The production strain from which the glucose oxidase food enzyme is produced is a non-genetically modified strain of the filamentous fungus *P. chrysogenum*, designated as strain PGO 19-162. *P. chrysogenum* has a history of safe use in food production, as well as a history of safe use as a production organism used in the production of food enzymes, particularly glucose

oxidases, in Europe and in Japan. The production strain *P. chrysogenum* PGO 19-162 is non-pathogenic and does not produce any known mycotoxins. The production strain has been deposited in a recognised culture collection and also is appropriately stored and monitored at Shin Nihon.

A Hazard Analysis and Critical Control Points (HACCP) plan is in place for the manufacture of the glucose oxidase food enzyme. The established quality control procedures ensure pure culture and optimum enzyme productivity conditions during fermentation. Manufacture of the food enzyme includes a purification process to ensure absence of microbiological contamination and removal of the production strain. Furthermore, only those batches of the food enzyme that meet the established specifications are released.

Reaction and Fate in Food

The glucose oxidase food enzyme is used during food and beverage processing to reduce the residual glucose and/or oxygen content during the production of a variety of foods and beverages. The enzymatic reaction catalysed by glucose oxidase leading to this effect is the oxidation of β-D-glucose to D-glucono-1,5-lactone (D-glucono-δ-lactone) in the presence of molecular oxygen, which, at the same time, converts oxygen to hydrogen peroxide. The enzyme therefore performs its catalytic function directly on β-D-glucose molecules present in various food matrices during processing of the foods. D-Glucono-1,5-lactone is subsequently hydrolysed to gluconic acid by non-enzymatic means. No safety concerns are raised with respect to the D-glucono-1,5-lactone and gluconic acid products. With regards to hydrogen peroxide, the level of this substance that would be produced under the intended conditions of use of the glucose oxidase food enzyme would be equivalent to the levels produced by the current uses of glucose oxidase derived from other sources, which are considered safe. In addition, the enzyme will be used only at the level required to achieve the intended effect, limiting any excessive production of hydrogen peroxide. Furthermore, glucose oxidase is often used in conjunction with catalase, which catalyses the breakdown of hydrogen peroxide to oxygen and water.

Any residual glucose oxidase following its addition during food processing will typically be heat-denatured; thus, the enzyme would have no technological effect on the final foods as consumed in these instances. The use of glucose oxidase in certain applications (*e.g.*, use in soft drinks), however, would not be followed by an inactivation or removal step, and therefore, the enzyme may continue to perform a technological function in the final food in the presence of glucose and when exposed to oxygen. Such uses of glucose oxidase, however, consist of well-established and typical applications in the food industry.

Proposed Conditions of Use in Food Manufacturing and the Proposed Maximum Use Levels

The intended technological need of the glucose oxidase food enzyme is to catalyse the oxidation of β-D-glucose to D-glucono-1,5-lactone (D-glucono-δ-lactone) in the presence of molecular oxygen, which, at the same time, converts oxygen to hydrogen peroxide during

the production of a variety of foods and beverages. The enzyme is used for preservative and stabilising purposes and for improving the texture of such foods as breads and other baked products. The technological need for glucose oxidase is established in the food industry and cannot be sufficiently met with the use of other enzymes or by other means.

The maximum levels of use of the glucose oxidase food enzyme used in the production of foods and beverages are 30 and 10 mg TOS/kg, respectively. The intended uses of the food enzyme are consistent with existing applications of glucose oxidase in food processing world-wide. The food enzyme is used only at the level required to achieve the intended effect.

The theoretical maximum daily intake (TMDI) calculated for the glucose oxidase food enzyme using Budget Method assumptions for exposure was 0.63 mg TOS/kg body weight/day from both solid foods and non-milk beverages.

TOXICOLOGICAL DATA

The core set of toxicological tests have been performed on Shin Nihon's glucose oxidase food enzyme produced with *P. chrysogenum* PGO 19-162 in accordance with EFSA's guidance on food enzyme dossiers. The food enzyme was non-mutagenic/non-genotoxic in all genotoxicity tests. In the repeated-dose 90-day oral toxicity study conducted in rats, the no-observed-adverse-effect level (NOAEL) for the oral toxicity of the glucose oxidase food enzyme in rats was determined to be 193 mg TOS/kg body weight/day, the highest dose tested. A large margin of safety exists between the NOAEL for the glucose oxidase food enzyme and the estimated maximum potential daily intakes on a TOS basis. No toxicity concerns are therefore raised with respect to the conditions of use of the glucose oxidase food enzyme.

No concerns for allergenicity are raised as the potential for glucose oxidase produced *P. chrysogenum* PGO 19-162 to cross-react with known allergens is low. Additionally, there is no evidence from the available scientific literature or from the history of use of glucose oxidase enzyme preparations formulated with Shin Nihon's glucose oxidase food enzyme (produced with *P. chrysogenum* PGO 19-162) in Japan indicating allergenicity to the enzyme in consumers. Based on this information, no evidence exists that might indicate that glucose oxidase produced by *P. chrysogenum* PGO 19-162 would produce an allergenic response following use in food processing.