

**FORM FOR THE SUBMISSION OF SUBSTANCES TO BE EVALUATED BY JECFA**

*In completing this form, only brief information is required. The form may be retyped if more space is needed under any one heading provided that the general format is maintained.*

<b>Name of Substance(s):</b>	Protease Aqualysin 1 from <i>Thermus aquaticus</i> produced by <i>B. subtilis</i> , strain LMGS 25520
<b>Question(s) to be answered by JECFA</b>	Safety evaluation when used as a processing aid

1. Proposal for inclusion submitted by:

The Belgian Federal Public Service Health, Food Chain Safety and Environment  
Place Victor Horta 40 box 10  
1060 Brussels/Sint-Gillis  
Belgium  
E-mail: apf.food@health.belgium.be

2. Name of substance; trade name(s); chemical name(s):

Name: Protease Aqualysin 1 from *T. aquaticus* produced by *B. subtilis*, strain LMGS 25520  
IUBMB name: EC 3.4.21.111  
Tradename: Premix X-220 (Main commercial name)  
chemical names: Aqualysin 1, Caldolysin

3. Names and addresses of basic producers

Puratos NV- site Beldem  
Rue Bourrie 12  
B-5300 Andenne  
Belgium  
Tel. no: +32 8582 3250  
Fax no: 32 8582 3260

4. Has the manufacturer made a commitment to provide data?

Puratos NV commits to provide data to support the proposal for the inclusion of Protease Aqualysin 1 in the list of substances to be evaluated by the JECFA.

5. Identification of the manufacturer that will be providing data (Please indicate contact person):

Name: Puratos NV  
Address: Industrialaan 25  
Postal code and City: B-1702 Groot-Bijgaarden  
Country: Belgium  
Tel. no: +32 2481 4444  
Fax no: +32 2466 2581  
Contact person: Bas Verhagen  
Tel: +32 2481 4551  
E-mail: BVerhagen@puratos.com

6. Justification for use:

The food enzyme catalyses, i.e. accelerates, hydrolyses of the peptide bonds that link amino acids together in the polypeptide chains forming the proteins.

The addition of protease Aqualysin 1 provides the following benefits, of interest during the production of bakery products:

- Faster dough development upon mixing

- Better dough machinability
- Reduced dough rigidity which results in processing tolerance
- Improved dough's structure and extensibility during the shaping or moulding step
- Uniform shape of the bakery product
- Regular batter viscosity, beneficial in the production process for e.g. waffles, pancakes and biscuits
- Improved short-bite of certain products like hamburger breads.

7. Food products and food categories within the GSFA in which the substance is used as a food additive or as an ingredient, including use level(s)

The enzyme preparation is used as a processing aid during production of bakery products., it is therefore not added to the final food product. The food enzyme is denatured during the baking process. Therefore it cannot have any technological function anymore in final baked foods.

Food enzyme preparations are used by food manufacturers according to the *Quantum Satis* principle, which means that food manufacturers will typically fine-tune the enzyme dosage based on a dose range recommended by the enzyme supplier. The recommended dose ranges in baking processes is 2300 - 12000 mU per kg raw material (flour).

8. Is the substance currently used in food that is legally traded in more than one country? (please identify the countries); or, has the substance been approved for use in food in one or more country? (please identify the country(ies))

The use in food of the Protease Aqualysin 1 from *T. aquaticus* produced by *B. subtilis*, strain LMGS 25520 is officially approved in France, Canada and the US.

Moreover, the enzyme is legally traded and the use in food is legal in many countries worldwide, including in the EU.

Finally, a new dossier has also been submitted in the EU under Regulation (EC) No 1332/2008, and is currently under review by the EFSA.

9. List of data available (please check, if available)

**Toxicological data**

The *B. subtilis* production strain is from a safe lineage that has been tested according to the criteria laid out in Pariza and Johnson (2001<sup>1</sup>). The safety of *B. subtilis* as a production microorganism has been assessed by EFSA (2007<sup>2</sup>) and has been accorded Qualified Presumption of Safety (QPS) status. A review of the literature by the US EPA (1997<sup>3</sup>) failed to reveal the production of metabolites of toxicological concern by *B. subtilis*. Nonetheless, to comply with the various regulatory requirements in different countries, several toxicity experiments have been performed.

- (i) Metabolic and pharmacokinetic studies

Not applicable

- (ii) Short-term toxicity, long-term toxicity/carcinogenicity, reproductive toxicity, and developmental toxicity studies in animals and genotoxicity studies

The food enzyme has been subjected to a standard package of toxicological tests in line with internationally accepted guidelines (OECD/EU), with the following results:

Ames test:	No mutagenic activity under the given test conditions
Chromosomal aberrations:	No clastogenic activity under the given test conditions
90-day oral toxicity on rats:	The No Observed Adverse Effect Level (NOAEL) is 606 mg TOS/kg bw/day, which is the highest dose in the study.

<sup>1</sup> Evaluating the safety of microbial enzyme preparations used in food processing: update for a new century. Regulatory Toxicology and Pharmacology 33:173-186.

<sup>2</sup> Opinion of the Scientific Committee on a request from EFSA on the Introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA. The EFSA Journal 587, 1-16.

<sup>3</sup> <https://www.epa.gov/sites/production/files/2015-09/documents/fra009.pdf>.

In short, the food enzyme, object of the present dossier, was subjected to several toxicological studies to confirm its safety for consumers. The mutagenicity studies supported that the food enzyme does not have the potential to damage the genetic material of living organisms, including mammals. The oral toxicity study showed that the food enzyme does not exhibit signs of toxicity, up to doses that are several thousand times higher than those which are consumed via food.

- (iii) Epidemiological and/or clinical studies and special considerations

Not applicable

- (iv) Other data

None

#### **Technological data**

- (i) Specifications for the identity and purity of the listed substances (specifications applied during development and toxicological studies; proposed specifications for commerce)

The food enzyme Aqualysin complies with the JECFA specifications for chemical and microbiological purity of food enzymes (FAO/WHO Monographs 3, 2006) and with the French purity criteria of enzymes (AR 19/10/2006). Neither the production strain nor antibiotic resistance genes are present in the final product. Moreover, no presence of biologically active DNA has been shown.

The Aqualysin 1 described in this dossier is manufactured in accordance with current Good Manufacturing Practice for Food (GMP) and the principals of Hazard Analysis of Critical Control Points (HACCP) and in line with Food Hygiene Regulation (EC) No 852/2004 and Regulation (EC) No 178/2002.

- (ii) Technological and nutritional considerations relating to the manufacture and use of the listed substance

Aqualysin 1 from *B. subtilis* is a protein used at very low dosage. Therefore, it has no nutritional impact on the final baked products. The food enzyme is denatured during baking. As other food proteins, the enzyme may be hydrolyzed into its constitutive amino acids. Products of the protease hydrolysis are natural constituents of the cereals based foods and no anti nutritional effects could result of the degradation of proteins by the protease food enzyme. Use of Aqualysin 1 from *B. subtilis* in food (e.g. baking applications) doesn't alter the nutritional composition nor modify nutritional value of foodstuffs.

#### **Intake assessment data**

- (i) Levels of the listed substance used in food or expected to be used in food based on technological function and the range of foods in which they are used

The food enzyme object of this dossier is typically used in the manufacturing of bakery products such as, but not limited to, bread, biscuits, steamed bread, cakes, pancakes, tortillas, wafers and waffles.

Based on the maximum recommended use level for the enzyme per raw material (flour) and the average amount of flour used for baking, the maximum level of TOS in the final food will be 151.58 mg TOS/ kg food.

- (ii) Estimation of dietary intakes based on food consumption data for foods in which the substance may be used.

The total Theoretical Maximum Daily Intake (TMDI) can be calculated on basis of the maximal values found in baked foods, multiplied by the maximum consumption of baked foods/kg body weight/day. Based on the recommended use levels, the amounts of the respective ingredients that end up in the final foods and the amount of produce consumed by the high end consumers, the TMDI of the food enzyme Aqualysin 1 from *B. subtilis* was calculated to be 622.9 µg TOS/kg body weight/day.

It should be stressed that this Total TMDI is based on conservative assumptions and represents a highly exaggerated value.

***Other information (as necessary/identified)***

None

10. Date on which data could be submitted to JECFA.

As soon as required.