INFORMATION ON THE SUBSTANCE TO BE EVALUATED BY JECFA

1. ***Proposal for inclusion submitted by:***

Danish Veterinary and Food Administration.

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

Substance: Lipase from *Aspergillus oryzae* expressing a modified gene from *Thermomyces lanuginosus*

Chemical name: Triacylglycerol lipase; CAS 9001-62-1, EC 3.1.1.3

3. ***Names and addresses of basic producers:***

Novozymes A/S
Krogshøjvej 36
DK-2880 Bagsværd
Denmark

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

Novozymes A/S commits to provide data to support the proposal for inclusion of the lipase in the list of substances to be evaluated by JECFA.

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

Novozymes A/S
Krogshøjvej 36
DK-2880 Bagsværd
Denmark

Attn.: Peter Hvass
phva@novozymes.com
+45 4446 3610

6. ***Justification for use:***

The lipase enzyme preparation is used as a processing aid during food manufacture for hydrolysis of lipids during processing of lipid-containing foods, e.g. in order to improve dough strength and stability in baking and other cereal based processes.

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

The enzyme preparation is not added to final foodstuffs but used as a processing aid during food manufacturing. The lipase is used in baking and other cereal based food processes.

The lipase is used at the minimum dosage necessary to achieve the desired enzymatic reaction. The range of dosage recommended for the lipase is up to 2200 LU per kilogram flour.

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

The enzyme is marketed under the trade name of Lipopan Xtra which was approved in Denmark in 2008. The enzyme has also been positively evaluated by a number of regulatory authorities, resulting in inclusion on various positive lists, e.g. in France, Mexico, Brazil.

9. ***List of data available (please check, if available)***
   Toxicological data
   (i) Metabolic and pharmacokinetic studies
   (ii) Short-term toxicity, long-term toxicity/carcinogenicity, reproductive toxicity, and developmental toxicity studies in animals and genotoxicity studies
   (iii) Epidemiological and/or clinical studies and special considerations
   (iv) Other data

The following food toxicity program according to the EFSA Guideline[i] has been performed:

- Test for mutagenic activity (Ames Test)
- *In vitro* micronucleus
- 13 weeks oral toxicity study in rats

The main conclusions of the safety studies can be summarized as follows:
The lipase preparation showed no mutagenic activity by testing in a bacterial reverse mutation assay (Ames Test) and did not induce micronuclei in cultured human peripheral blood lymphocytes *in vitro*.

The lipase preparation did not result in treatment-related adverse effects when administered to rats for 13 weeks, and the overall No Observed Adverse Effect Level (NOAEL) is considered to be the highest administered dose, corresponding to 1080 mg TOS/kg body weight (bw)/day.

The safety studies described above were all performed on liquid lipase enzyme concentrate produced in accordance with ordinary production procedure, omitting stabilization and standardization.

Aspergillus oryzae is generally considered to be a safe production organism with a long history of safe use for food ingredients. Furthermore, the production strain lacks the ability to produce relevant mycotoxins.

Technological data

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

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

The lipase enzyme preparation complies with the purity criteria recommended for enzyme preparations by Food Chemicals Codex (VIII online edition, 2012). In addition to this, the enzyme preparation also conforms to the General Specifications and Considerations for Enzyme Preparations Used in Food Processing (2006) as proposed by the Joint FAO/WHO Expert Committee on Food Additives in Combined Compendium of Food Additive Specifications.

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

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

The exposure assessment is performed according to the Budget Method (ILSI, 1997). The Budget Method assumptions represent a "maximum worst case" situation of human consumption, in which the enzyme would be used at its maximum recommended dosages in all processed food and not only in the baking and other cereal based food processes described above.

Overall, the human exposure to the lipase will be negligible because the enzyme preparation is used as a processing aid and in low dosages. It is also supposed that the totality of the food enzyme will end up in the final food. This assumption is exaggerated since the enzyme protein and the other substances resulting from the fermentation are diluted or removed in certain processing steps.

Therefore the safety margin calculation derived from this method is highly conservative.

Assumptions in the Budget Method

Solid food	The maximum energy intake over the course of a lifetime is 50 kcal/kg bw/day. 50 kcal corresponds to 25 g foods. Therefore, adults ingest 25 g foods per kg bw per day. Assuming that 50% of the food is processed food, the daily consumption will be 12.5 g processed foods per kg bw. Since the baking process on average results in 140 g of final baked product from 100 g of flour, it is further assumed that all processed food contains 70% flour = 8.75 g flour per kg bw per day.

Theoretical Maximum Daily Intake (TMDI) calculation

Solid Food:
The highest dosage is 2200 LU per kilogram flour. 2200 LU correspond to 20.05 mg TOS. Based on this, 8.75 gram flour based processed food will maximally contain 0.18 mg TOS.

The theoretical maximum daily intake (TMDI) of the enzyme by consumers is therefore 0.18 mg TOS/kg body weight/day.

Other information as necessary

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

August 2016

[i] Guidance of EFSA prepared by the Scientific Panel of Food Contact Material, Enzymes, Flavourings and Processing Aids on the Submission of a Dossier on Food Enzymes. The EFSA Journal (2009) 1305, 1-26