codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

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**Agenda Item 12** **CX/FA 07/39/18**
**February 2007**

**JOINT FAO/WHO FOOD STANDARDS PROGRAMME**

**CODEX COMMITTEE ON FOOD ADDITIVES**

**Thirty-ninth Session**

**Beijing, China, 24-28 April 2007**

# PRIORITY LIST OF FOOD ADDITIVES PROPOSED FOR EVALUATION BY JECFA (IN RESPONSE TO CL 2006/41-FA)

**The following comments have been received from the following Codex Members: Netherlands and U.S.A**

## Netherlands:

### 1. Proposal for inclusion submitted by:

Dr. Wieke Tas
Ministry of Health, Welfare and Sports
PO Box 20350
2500 EJ The Hague
The Netherlands
Phone: + 31 70 340 6365
Fax: + 31 70 340 5554
E-mail: jw.tas@minvws.nl

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

Compound: *Aspergillus niger* asparaginase expressed in *Aspergillus niger*.

Trade name: Preventase™

Chemical name(s):

- Systematic name : L-asparagine amidohydrolase
- Common name : Asparaginase
- Other names : asparaginase II; L-asparaginase; colaspase; elspar; leunase; crasnitin; alpha-asparaginase
- Enzyme Commission No. : 3.5.1.1
- CAS number : 9015-68-3

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

DSM Food Specialties
15 Rue des Comtesses
PO Box 50239
59472 SECLIN Cédex
FRANCE

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

DSM Food Specialties commits to provide data to support the proposal for inclusion of the asparaginase in the list of substances to be evaluated by JECFA.

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

DSM Food Specialties
15 Rue des Comtesses
PO Box 239
59472 Seclin Cédex
France
Attn.: Francois Strozyk
francois.strozyk@dsm.com
+33 320964514

**6. Justification for use :**

The asparaginase enzyme preparation is used as a processing aid during food production to convert asparagine to aspartic acid in order to reduce acrylamide formation.

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

The asparaginase enzyme preparation is intended to be used to reduce acrylamide formation during food production of L-asparagine- and carbohydrate-containing foods that are heated above 120°C, such as bread and other baked cereal-based products, baked or fried potato-based products and reaction flavors.

The commercial product, Preventase™, will be presented in two forms:

- one liquid standardized to an enzyme activity ranging from 2300 to 2600 ASPU/ml
- one granulated form standardized to an enzyme activity ranging from 9500 to 10500 ASPU/g

The dosage of Preventase™ in food production is typically less than 0.1%.

**8. Has the compound been approved for use in 2 or more countries (please identify the countries)?**

A US GRAS (Generally Recognized As Safe) Notification has been submitted in October 2006.

A submission of Preventase™ for approval in France has been submitted in January 2007.

**9. List of data (toxicology, metabolism, specifications) available:**

The production organism is from a safe strain lineage as described in the article of P. van Dijck et al[1] and the decision tree in Pariza and Johnson[2]. Nevertheless, to comply with various approval requirements in different countries world-wide, a full safety program as described in the SCF Guidelines[3] has been performed:

- 14-day dose range-finding/ feasibility study with an enzyme preparation of *Aspergillus niger* containing asparaginase activity in rats.
- Repeated-dose (13-week) oral toxicity study with an enzyme preparation of *Aspergillus niger* containing asparaginase activity in rats
- Oral prenatal developmental toxicity study with an enzyme preparation of *Aspergillus niger* containing asparaginase activity in rats
- Bacterial reverse mutation test with enzyme preparation of *Aspergillus niger*

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[1] Dijck, P.W.M. van, Selten, G.C.M., Hempenius, R.A., *On the safety of a new generation of DSM Aspergillus niger enzyme production strains*, Regulat. Toxicol. Pharmacol. 38:27-35 (2003)

[2] Pariza, M.W. and Johnson, E.A., *Evaluating the safety of microbial enzyme preparations used in food processing: Update for a new century*, Regulat. Toxicol. Pharmacol. 33:173–186 (2001)

[3] Guidelines for the presentation of data on food enzymes – *Opinion expressed on 11 April 1991* – Reports of the Scientific Committee for Food (27th series), 1992

- Chromosomal aberration test with an enzyme preparation of *Aspergillus niger* in cultured human lymphocytes.

The conclusion of the safety studies can be summarized as follows:

The enzyme preparation showed to be not mutagenic in a bacterial mutation assay (AMES test) or clastogenic in the chromosomal aberration assay with human lymphocytes in vitro. In addition the test item was evaluated in two studies with Wistar rats: a subchronic (90-day) feeding study and a prenatal developmental toxicity study. No adverse effects were observed at any level in these studies resulting in an overall No observed Adverse Effect Level (NOAEL) of 1157 mg/kg body weight/day. This corresponds to a Margin of Safety (NOAEL/estimated daily intake) in the range of 648 - 30772.

The test material used in these studies originates from one batch which was produced by the procedure used for the commercial preparation. After the purification step, the batch was spray dried to produce the final, non-stabilised test item.

The above mentioned safety studies were all performed on a liquid asparaginase enzyme concentrate, obtained in accordance with an ordinary production procedure, omitting stabilization and standardization.

Preventase™ complies with the purity criteria recommended for enzyme preparations as described in the Food Chemical Codex, 4th edition, 3rd supplement, 2001, as well as with the General Specifications and Considerations for Enzyme Preparations Used in Food Processing as laid down by the Joint FAO/WHO Expert Committee on Food Additives in 2006 (http://www.fao.org/ag/agn/jecfa-additives/search.html?lang=en)

Furthermore, it is documented that the production strain lacks the ability to produce relevant mycotoxins.

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

Before December 2007.

**U.S.A**

INFORMATION ON THE ADDITIVETO BE EVALUATED BY JECFA

Evaluation of Phospholipase C Enzyme Preparation

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

United States of America

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

Trade name – Purifine™

Chemical names – phospholipase C

PLC

**3. Name and address of basic producers:**

Diversa Corporation
4955 Directors Place
San Diego, CA 92121

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

The manufacturer, Diversa Corporation, is committed to providing data to JECFA.

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

Diversa Corporation
4955 Director's Place
San Diego, CA 92121

Contact:
Diane Shanahan
Assoc. Director, Regulatory Affairs
Ph. 858-526-5342
dshanahan@diversa.com

**6. Justification for use:**

Phospholipase C enzyme preparation is intended for use as a processing aid to hydrolyze phospholipids in the refining of soybean oil and other vegetable oils intended for use in foods. This hydrolysis results in the following benefits:

- Reduction in the amount of gum phospholipids and overall gum mass
- Reduction in the total phosphorus contained in the oil
- Reduction in the total mass of neutral oil entrained in the phospholipid gum
- Increase in the quantity of diacylglycerol contained in the oil
- Lower environmental impact because the enzyme is biodegradable and is inactivated during the process
- Lower usage of water and lower production of waste water
- Less need for bleaching clay and, therefore, less disposal of spent bleaching clay (which, incidentally, is combustible)

**7. Food products and food categories within the GSFA in which the compound is used, including use levels:**

GFSA Food Category: 02.1.2 Vegetable oils and fats.

Phospholipase C (PLC) enzyme preparation will be used as a processing aid to hydrolyze phospholipids in the refining of vegetable oils, such as soybean; corn; canola; rape; and sunflower. The enzyme preparation will be used at levels no higher than necessary to achieve the intended effect, generally in the range of 100 to 1000 grams of enzyme preparation per metric ton of oil, depending on the oil to be treated and the reaction conditions.

The first step in the refining process is known as “degumming” and it is during this step that the PLC enzyme preparation will be used. After the degumming reaction has been completed, subsequent steps in refining, including repeated washing of the oil with water, bleaching and deodorization, will remove any remaining enzyme residues. For these reasons, inactivated and denatured enzyme may be present, if at all, in the fully refined vegetable oil at levels close to or below the limit of detection (LOD=1ppb) of the available, highly-sensitive analytical method. The method of quantitation used to determine these residue levels was an enzyme-linked immunosorbent assay (ELISA).

**8. Has the compound been approved for use in 2 or more countries (please identify the countries)?**

Diversa’s phospholipase C enzyme preparation has successfully completed a GRAS notice review in the United States and is currently under review in Canada, France, and Argentina.

**9. List of data (toxicology, dietary exposure, specifications on chemical identity and purity, analytical methods) available:**

The list of toxicology data consists of:

- Genotoxicity (Ames assay, Mouse micronucleus assay, and Chromosomal aberrations in human lymphocyte assay),
- Oral toxicity (Acute oral toxicity in rats, 14-day dose range-finding oral toxicity in rats, and 90-day oral toxicity in rats),
- Worker safety studies (Acute nose-only inhalation in rats, Primary eye irritation, Primary dermal irritation, and Delayed contact hypersensitivity in guinea pigs)

- Dietary exposure, specifications on chemical identity and purity, and analytical methods are all available.

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

Data can be submitted to JECFA on or before November 30, 2007.