Annex 4

FORM ON WHICH INFORMATION ON THE COMPOUND TO BE EVALUATED BY JECFA IS PROVIDED

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.

Name of Compound(s):	**Phosphatidyl inositol-specific phospholipase C from a genetically modified strain of *Pseudomonas fluorescens***
Question(s) to be answered by JECFA *(kindly provide a brief justification of the request in case of re-evaluations)*	Safety evaluation when used as processing aid.

1. Proposal for inclusion submitted by:

Ministry of Health, Welfare and Sport
Nutrition, Health Protection and Prevention Department
Parnassusplein 5
2511 VX The Hague
P.O. box 20350
2500 EJ The Hague
The Netherlands
Tel: +31 703407132

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

Name of compound : Phosphatidyl inositol-specific phospholipase C from a genetically modified strain of *Pseudomonas fluorescens*

Trade names : Purifine SB4, Purifine SB5, Purifine 3G

Chemical names : Phosphatidyl inositol-specific phospholipase C (EC 3.1.4.11)

3. Names and addresses of basic producers:

DSM Food Specialties
15 Rue des Comtesses
PO Box 239
59472 Seclin Cédex
France
Tel: 33 320964545
Fax: 33 320964500

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

Yes.

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

Dr Mariella Kuilman
Regulatory Affairs Manager
DSM Food Specialties
PO Box 1
2600 MA Delft

The Netherlands
Tel: +31 (0) 15 2793592
Fax: +31 (0) 15 2793614
E-mail: Mariella.kuilman@dsm.com

6. Justification for use:

The enzyme Phosphatidyl inositol-specific phospholipase C hydrolyzes phosphatidylinositol present in vegetable oil resulting in the formation of diacylglycerol and phophorylinositol. Phosphatidylinositol can negatively impact taste, color and stability of the vegetable oil while diacylglycerol and phosphorylinositol do not have that effect. The removal of phosphatidylinositol with the help of this enzyme preparation may be of benefit in oil refining to give a higher oil yield at lower cost and a lower environmental impact compared to conventional refining methods.

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 used as processing aid in oil degumming in accordance with current Good Manufacturing Practice (cGMP). The dosage of the enzyme varies between 30 to 300 grams of enzyme preparation per metric ton of oil depending on the specific application.

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 preparation containing phosphatidyl inositol-specific phospholipase C from a genetically modified strain of *Pseudomonas fluorescens* is authorized/allowed in the following countries:

- USA : GRN 574
- Argentina
- Canada
- EU (except Denmark, France and Spain)

Besides, the enzyme preparation containing phosphatidyl inositol-specific phospholipase C from a genetically modified strain of *Pseudomonas fluorescens* is under evaluation in the following countries:

- Brazil
- Mexico

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

The production organism is from a safe strain as described in the decision tree in Pariza and Johnson, 2001[1]. However, to accommodate various registration requirements in different countries world-wide, a full toxicity program for food enzymes has been performed according to the OECD guidelines and EFSA guidelines for the evaluation of food enzymes[2].

[1] Pariza MW, Johnson EA; Evaluating the safety of microbial enzyme preparations used in food processing: update for a new century; Regul Toxicol Pharmacol 2001 Apr;33(2):173-86.
[2] 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, 1305, 1-26.
http://www.efsa.europa.eu/en/efsajournal/doc/1305.pdf

Toxicological data

(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 following studies have been conducted in accordance with internationally accepted guidelines (OECD/EU):

- Test for mutagenic activity (Ames Test)
- Chromosomal aberration test, *in vivo*
- 13 weeks oral toxicity in rats

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

The enzyme from genetically modified *Pseudomonas fluorescens* shows no mutagenic and clastogenic activity.

13 weeks oral administration of the enzyme to rats did not cause any dose related findings. Therefore, the highest dose administered, 2000 mg test substance/kg body weight/day which is 1838 mg TOS/kg body weight/day is considered as the NOAEL.

(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 compounds (specifications applied during development and toxicological studies; proposed specifications for commerce)

The product conforms to the General Specifications and Considerations for Enzyme Preparations Used in Food Processing as prepared by the Joint FAO/WHO Expert Committee on Food Additives at its sixty-seventh meeting for publication in FAO JECFA Monographs 3 (2006) and to the acceptance criteria, impurity limits, other test and other requirements for enzyme preparations listed in the Food Chemicals Codex, 9th edition.

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

The enzyme preparation from genetically modified *Pseudomonas fluorescens* will be used as processing aid in oil degumming. In degumming of vegetable oils, the enzyme will end up in water phase whereas the oil is the product phase that will end up in final food applications. Hence, no enzyme activity remains in the final food. The use of the enzyme preparation as processing aid has no influence on the nutritional properties of the final product.

Intake assessment data

(i) Levels of the listed compound 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 dosage of the enzyme varies between 30 to 300 grams of enzyme preparation per metric ton of oil depending on the specific application.

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

In the case of oil degumming, the TOS will not end up in the final product since the enzyme TOS will end up completely in the water phase whereas the oil phase is the product of interest.

Other information as necessary

None

10. Date on which data could be submitted to JECFA

As soon as necessary.