CODEX ALIMENTARIUS COMMISSION

Food and Agriculture Organization of the United Nations

World Health Organization

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Agenda Item 7 **CX/FA 17/49/13 Add.1**

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JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON FOOD ADDITIVES

Forty-ninth Session

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PROPOSALS FOR ADDITIONS AND CHANGES TO THE PRIORITY LIST OF SUBSTANCES PROPOSED FOR EVALUATION BY JECFA

Comments of Egypt, Japan and Russian Federation

Egypt

General Comment:

Egypt has procedural concerns about some recommendations in the EWG report. For example, the statements made questioning the safety of amylases, proteases, and lipases contradict the safety evaluations made by JECFA as all have been found safe for use. We also question the proposed deletion of additives that have adopted provisions in the GSFA (natamycin/pimaricin and nisin) as it is contrary to the CCFA procedures and beyond the role of the EWG and Egypt didn't support nor modification or deletion of those items due to the technological usage under the approved safe limits, Egypt wishes that the in-session working group on the INS will clarify these concerns.

Specific Comment:

Egypt adopt the position of IFAC (International Food Additives) & ELC (Federation of European Specialty Food Ingredients Industries) and other several member states and the position based on the following points;

1- Natamycin/Pimaricin and Nisin are widely used in products in international trade to avoid any trade barriers,

2- Those preservatives possess anti-microbial activity against a wide range of Gram-positive bacteria and their spores which causes food spoilage, and especially inhibits the heat-resistant bacilli; such as B. Stearothermophilus, CI. Butyricum, Clostridia botulinum growthand L. Monocytogenes and thereby helps to extend product shelf life and to ensure food safety.

Japan

Name of Substance(s):	Microbial collagenase from *Streptomyces violaceoruber* expressed in *S. violaceoruber*
Question(s) to be answered by JECFA *(Provide a brief justification of the request in case of re-evaluations)*	Safety evaluation when used as a processing aid and establishment of specifications

1. Proposal for inclusion submitted by: JAPAN
2. Name of substance; trade name(s); chemical name(s):

   Name of substance: microbial collagenase from *Streptomyces violaceoruber* expressed in *S. violaceoruber*

   Trade names: DENAZYME CPO PEPRICH XPP-051

   Chemical name: microbial collagenase (EC 3.4.24.3)
3. Names and addresses of basic producers:

   Nagase ChemteX Corporation

   1-52 Osadano-cho, Fukuchiyama, Kyoto, 620-0853, Japan

(Attn: Mr. Yasunori Makise, General Manager, Bio Chemicals Department)

Tel: +81-773-27-5801

Fax: +81-773-27-2040

E-mail: yasunori.makise@ncx.nagase.co.jp

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):

Nagase ChemteX Corporation

1-52 Osadano-cho, Fukuchiyama, Kyoto, 620-0853, Japan

(Attn: Mr. Kensaku Uzura, Quality Assurance Section, Bio Chemicals Department)

Tel: +81-773-27-5803

Fax: +81-773-27-2040

e-mail: kensaku.uzura@ncx.nagase.co.jp

6. Justification for use:

The collagenase preparation is used in the processing of meat and sausage casing. When applied in meat processing, collagenase reduces connective tissue toughness by selectively hydrolyzing collagen and improves meat tenderness.

The collagenase preparation is also used in the production of collagen hydrolysates which is used as food ingredients in food supplements.

The benefits of using collagenase are as follows;

- In the processing meat and sausage casing

  As collagenase selectively hydrolyzes collagen which is the main protein constituting connective tissue in meat, it is possible to tenderize meat without affecting the taste.

- In the production of collagen hydrolysates

  As collagenase selectively catalyzes the hydrolysis of peptide bond in collagen (no unintended reaction products are generated), the natural hydrolysate suitable for food material, which is used in various foods safely is obtained.

In the both applications mentioned above, heating treatment, such as cooking or sterilization, is carried out after each enzyme reaction. Collagenase activity is inactivated during the heating treatment, e.g. cooking at 60 to 100C for 30 to 180min in the processing meat and sausage casing, and sterilization at 90C for 10 to 60 min in the production of collagen hydrolysates. Consequently, the collagenase does not exert any (unintentional) enzymatic activity in the final food. No effect can occur on other food constituents such as lipids and proteins.

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 food categories within GSFA are; Meat and meat products, including poultry and game (FC 08.0), Edible casings (e.g. sausage casings) (FC 08.4) and Protein products other than from soybeans (FC 12.10).

The ranges of the use levels of collagenase (mg TOS / kg food) can be provided. Some examples of the use levels are indicated in the table in “intake assessment data” section.

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))

Currently, this microbial collagenase preparation is only approved in Japan. In Europe and France, the dossier is under evaluation.

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

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

This microbial collagenase preparation has been subjected to toxicological tests as follows; (in accordance with internationally accepted guidelines (OECD/Japan))

a) Ames test (Test for mutagenic activity)

b) Chromosomal aberrations

c)Micronucleus test

d) Acute toxicity test on rats

e) 90-day oral toxicity on rats

(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)

Its specifications comply with the *General Specifications and Considerations for Enzyme Preparations used in Food Processing* in FAO JECFA Monograph 3 (2006). The details are described below;

1) Produced by non-pathogenic and nontoxigenic strain

2) Produced in accordance with good food manufacturing practice

3) Comply with the purity in the following;

3-1) Lead: Not more than 5 mg/kg

3-2) Microbiological criteria

3-2-1) Salmonella species: absent in 25g of sample

3-2-2) Total coliforms: not more than 30 per gram

3-2-3) Escherichia coli: absent in 25g of sample

3-2-4) Antimicrobial activity: absent in preparation

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

The collagenase preparation is used in the processing of meat and sausage casing. When applied in meat processing, collagenase reduces connective tissue toughness by selectively hydrolyzing collagen and improves meat tenderness. The collagenase preparation is also used in the production of collagen hydrolysates which is used as food ingredients in food supplements. During heating step of processing of meat and sausage casing, and during sterilization step in the process of collagen hydrolysate, the enzyme is inactivated and no residual in the final product. In general, the collagenase does not exert any (unintentional) enzymatic activity in the final food. No effect can occur on other food constituents such as lipids and proteins. Thus, the use of the enzyme preparation has no influence on the nutritional properties of final food.

The benefits of using collagenase are as follows;

- In the processing meat and sausage casing

  As collagenase selectively hydrolyzes collagen which is the main protein constituting connective tissue in meat, it is possible to tenderize meat without affecting the taste.

- In the production of collagen hydrolysates

As collagenase selectively catalyzes the hydrolysis of peptide bond in collagen no unintended reaction products are generated), the natural hydrolysate suitable for food material, which is used in various foods safely, is obtained.

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

This collagenase preparation is intended to use in the processing of meat and sausage casing, and also in the production of collagen hydrolysates.

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 Nagase.

The intended use levels are;

36.36 mg TOS/kg food in meat processes

1566.54 mg TOS/kg food in collagen hydrolysate production

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

Food intake estimates were performed using the EU Datex database and considering the corresponding categories. These calculations give maximum dietary exposures for the “other children” category, that is, 0.16 and 0.86 mg TOS/kg body weight/day at the mean and the 95th percentile, respectively.

However, it must be emphasized that these food exposures are based on conservative assumptions and represent a highly exaggerated value based on following assumptions;

- It is assumed that ALL food producers use the food enzyme at the highest recommended level.
- It is assumed that consumers only eat foodstuffs processed with the enzyme preparation (aggregated/cumulative exposures)
- For the estimates using the Datex database, it is assumed that people intakes are the maximum available in these databases.

Other information (as necessary/identified)

None

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

Immediately

Name of Substance(s):	5'-Deaminase from *Streptomyces murinus*
Question(s) to be answered by JECFA *(Provide a brief justification of the request in case of re-evaluations)*	Safety evaluation when used as processing aid and establishment of specifications.

1. Proposal for inclusion submitted by: Japan
2. Name of substance; trade name(s); chemical name(s):

   Name of substance : 5'-Deaminase from *Streptomyces murinus*

   Trade names : Deamizyme T “Amano” (main commercial name)

   Chemical names : AMP deaminase (EC 3.5.4.6)
3. Names and addresses of basic producers:

   Amano Enzyme Inc.

   2-7, 1-Chome, Nishiki, Naka-ku, Nagoya, Aichi, 460-8630, Japan

   Tel: +81 (0)52-211-3032

   Fax: +81 (0)52-211-3054

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):

Amano Enzyme Inc.

2-7, 1-Chome, Nishiki, Naka-ku, Nagoya, Aichi, 460-8630, Japan

(Attn: Mr Tomonari Ogawa, Director, Quality Assurance Division)

Tel: +81 (0)52-211-3032

Fax: +81 (0)52-211-3054

e-mail: tomonari_ogawa@amano-enzyme.com

6. Justification for use:

The 5'-Deaminase is used in the processing of yeast and like products. When applied in that processing, 5'-Deaminase increases flavour component (umami) by deaminating adenosine monophosphate and improves flavour.

The benefits of using 5'-Deaminase is as follows;

As 5'-Deaminase deaminates AMP, AMP is converted into IMP.

During the lysis phase of yeast cells, four kinds of ribonucleotides, guanosine monophosphate (GMP), adenosine monophosphate (AMP), cytidine monophosphate (CMP) and uridine monophosphate (UMP) are formed by the breakdown of RNA. Among them, GMP and AMP contribute to umami taste. GMP provides the umami taste from the beginning. On the other hand, AMP itself is tasteless but it can be converted to tasty component, IMP by deaminating activity of 5'-Deaminase. As a result, flavour component in the lysate is increased by using this enzyme.

Therefore, it is possible to enhance the flavour of yeast and like products.

In the application mentioned above, heating treatment is carried out after the enzyme reaction. Consequently, the phosphodiesterase does not exert any (unintentional) enzymatic activity in the final food. No effect can occur on other food constituents such as carbohydrates, lipids and proteins.

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):

5'-Deaminase is used as processing aid in the products fallen within the Food Category 12.8 Yeast and like products in accordance with current Good Manufacturing Practice (cGMP). The dosage of the enzyme varies from 25 to 500 mg TOS/kg yeast extract.

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))

Currently, this 5'-Deaminase preparation is only approved in Japan. In Europe, the dossier is under evaluation.

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

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/Japan) and do not any concerns:

- A 13-week oral toxicity study in rats
- Bacterial reverse mutation test (Ames test)
- Chromosomal aberration test (*in vitro*)

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

The safety of 5'-Deaminase is assessed in a battery of toxicology studies investigating its genotoxic and systemic toxicity potential. Daily administration of the enzyme by gavage for 91 continuous days did not result in overt signs of systemic toxicity. Therefore, the highest dose administered, 1755 mg TOS/kg body weight/day, is considered as the NOAEL. A battery of genotoxicity assays was conducted and under the conditions of these assays the enzyme is not a mutagen or a clastogen.

(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)

5'-Deaminase 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, 10th edition. The details are described below;

1) Produced by non-pathogenic and nontoxigenic strain

2) Produced in accordance with good food manufacturing practice

3) Comply with the purity in the following;

3-1) Lead: Not more than 5 mg/kg

3-2) Microbiological criteria

3-2-1) Salmonella species: absent in 25g of sample

3-2-2) Total coliforms: not more than 30 per gram

3-2-3) Escherichia coli: absent in 25g of sample

3-2-4) Antimicrobial activity: absent in preparation

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

5'-Deaminase is used in the production of yeast extract, which is used as the key ingredient in seasonings, soups, sauces and gravies.

The enzyme converts the AMP into IMP in the lysate or extract of yeast by deamination.

The action of the enzyme takes place at the early phase of the process. After that, the enzyme is inactivated during inactivation or sterilization step by high temperature. Therefore, no enzyme activity remains in the final food. Thus the use of the enzyme as processing aid has no influence on the nutritional properties of the final food.

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 dosage of the enzyme varies from 25 to 500 mg TOS/kg yeast extract and assuming that the maximum dose of yeast extract in food is 2%, the amount of TOS in the final food will be 0.5-10 mg TOS/kg.

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

Based on the conservative calculation by means of the Budget method, assuming that the daily intake of processed foods is 50% of the total solid food intake, i.e. 0.0125 kg/kg bw/day and that the daily intake of soft drinks is 25% of the total beverages intake, i.e. 0.025 l/ kg bw/day, and calculating on basis of the maximal values found in food and beverage, the theoretical total daily intake will be 0.375 mg TOS/kg bw/day.

Other information (as necessary/identified) – None.

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

Immediately

Name of Substance(s):	Lipase from *Mucor javanicus*
Question(s) to be answered by JECFA *(Provide a brief justification of the request in case of re-evaluations)*	Safety evaluation when used as processing aid and establishment of specifications.

1. Proposal for inclusion submitted by: Japan
2. Name of substance; trade name(s); chemical name(s):

   Name of substance : Lipase from *Mucor javanicus*

   Trade names : Lipase MH “Amano”10SD (main commercial name)

   Chemical name : Triacylglycerol lipase (EC3.1.1.3)
3. Names and addresses of basic producers:

   Amano Enzyme Inc.

   2-7, 1-Chome, Nishiki, Naka-ku, Nagoya, Aichi, 460-8630, Japan

   Tel: +81-(0)52-211-3032

   Fax: +81-(0)52-211-3054
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):

   Amano Enzyme Inc.

   2-7, 1-Chome, Nishiki, Naka-ku, Nagoya, Aichi, 460-8630, Japan

   (Attn:Tomonari Ogawa, Director, Quality Assurance Division)

   Tel: +81-(0)52-211-3032

   Fax: +81-(0)52-211-3054

   e-mail: tomonari_ogawa@amano-enzyme.com
6. Justification for use:

The Lipase catalyses the hydrolysis of short, medium and long-chain fatty acids at 1, 2 and 3 positions of tri-, di- and monoglycerides (1 and 3 positions are preferent). The enzyme is used in the processing dairy, baking and egg products.

The benefits of using lipase in these applications are as follows;

- In the processing dairy products:

As this lipase hydrolyzes milk fats, free fatty acids are released. The fatty acids are one of the principal milk flavour component. Thus the enzyme improves savoury flavour of the final products.

- In the processing the baking products:

As this lipase hydrolyses lipids already present in the flour or added, fatty acid and monoglycerides are released. The monoglycerides unites with starch (amylose and amylopectin) strongly, and forms the complex in dough and the water absorption of starch is supressed. Resulted surplus water is utilized for construction of gluten structure and good extensibility is obtained. By this effect, gas retention capacity of the dough is increased and the finally, the volume and softness of the final products are improved.

- In the processing egg products:

As this lipase hydrolyses lipid in egg yolk, foamability of egg white is improved. In the industrial egg white production, slight amounts of yolk contamination causes negatively impact of its important function, foamability. This negative impact of egg yolk is originated from lipids in it, because low surface tension substances like lipids suppress the foaming property. Therefore, the characteristic of foaming of egg white is improved by hydrolyzing of lipid in egg yolk.

In the all applications mentioned above, heating treatment is carried out after the enzyme reaction.

Consequently, the lipase does not exert any (unintentional) enzyme activity in the final food. No effect can occur on other constituents such as carbohydrates and proteins.

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 food categories within the GSFA are;

Dairy products and analogues:

FC01.4 Cream (plain) and the like

FC01.5 Milk powder and cream powder and powder analogues (plain)

FC01.6 Cheese and analogues

FC01.7 Dairy-based desserts (e.g. pudding, fruit or flavoured yoghurt),

Bakery wares:

FC07.1 Bread and ordinary bakery wares

Eggs and egg products:

FC10.2 Egg products

The range of the use levels of the enzyme (mg TOS / kg food) can be provided. An example is indicated in “Intake assessment data” section.

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))

Currently, this lipase preparation is approved in Japan, Canada, Australia / New Zealand and Brazil. In Europe, the dossier is under evaluation.

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

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/Japan) and do not any concerns:

- A 13-week oral toxicity study in rats
- Bacterial reverse mutation test (Ames test)
- Chromosomal aberration test *(in vitro*)

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

The safety of the enzyme is assessed in a battery of toxicology studies investigating its genotoxic and systemic toxicity potential. Daily administration of the enzyme by gavage for 91 continuous days did not result in overt signs of systemic toxicity. Therefore, the highest dose administered, 20 mL /kg body weight/day (correspond to 784mgTOS/kg body weight/day), is considered as the NOAEL. A battery of genotoxicity assays was conducted and under the conditions of these assays the enzyme is not a mutagen or a clastogen.

(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 enzyme 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, 10th edition. The details are described below;

1) Produced by non-pathogenic and nontoxigenic strain

2) Produced in accordance with good food manufacturing practice

3) Comply with the purity in the following;

3-1) Lead: Not more than 5 mg/kg

3-2) Microbiological criteria

3-2-1) Salmonella species: absent in 25g of sample

3-2-2) Total coliforms: not more than 30 per gram

3-2-3) Escherichia coli: absent in 25g of sample

3-2-4) Antimicrobial activity: absent in preparation

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

Lipase is used in the process of dairy processing, baking and egg processing.

Dairy processing: The action of the enzyme takes place at the early phase of the process. The enzyme hydrolyses the lipid in dairy raw material and improves the flavour by the increment of free fatty acids that are one of the principal flavour agents. After that, the enzyme is inactivated at either inactivation or sterilization step by heating. Therefore, no enzyme activity remains in the final food.

Baking: By addition of this enzyme in dough mixing step, lipids already present in the flour or added are converted into free fatty acids and monoglycerides. The monoglycerides has emulsifying effect, so it works as good dough conditioners. These natural emulsifiers caused dough stability and elastic increase and as a result, volume and softness of bread are increased. After that, this lipase protein is denatured by heat during the baking or steaming step.

Egg processing: After the separation of egg white and egg yolk, the enzyme is used to egg white to improve the foamability by the elimination of the fat derived from contaminated egg yolk.

After that, the enzyme is inactivated at the sterilization / powderization step by high temperature. Therefore, no enzyme activity remains in the final food.

In all processes, the enzyme is denatured at (or before) the end of the food manufacturing process. Thus, the use of the enzyme has no influence on the nutritional properties of final food.

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

Dairy products and analogues: The dosage of the enzyme varies from 30 to 3016 mg TOS/kg raw material and assuming that the maximal dose of dairy hydrolysates in final food is 2%, the amount of TOS in the final food will be 0.6 - 60 mg TOS/kg.

Bakery wares: The dosage of the enzyme varies from 3 to 18 mg TOS/kg flour and assuming that 1kg of dough results in 1.4kg of bread, therefore, the percentage of raw material/final food is 71%. The amount of TOS in the final food will be 2 - 13 mg TOS/kg.

Eggs and egg products: The dosage of the enzyme varies from 3 to 12 mg TOS/kg egg white and assuming that the maximal dose of egg white used in final food is 30%, the amount of TOS in the final food will be 0.9 - 4 mg TOS/kg.

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

Based on the conservative calculation by means of the Budget method, assuming that the daily intake of processed foods is 50% of the total solid food intake, i.e. 0.0125 kg/kg bw/day and calculating on basis of the maximal values found in food (in the above cases dairy products), the daily intake will be 0.75 mg TOS/kg bw/day.

Other information (as necessary/identified)-None

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

Immediately

Name of Substance(s):	Phosphodiesterase from *Penicillium citrinum*
Question(s) to be answered by JECFA *(Provide a brief justification of the request in case of re-evaluations)*	Safety evaluation when used as processing aid and establishment of specifications.

1. Proposal for inclusion submitted by: Japan
2. Name of substance; trade name(s); chemical name(s):

   Name of substance : Phosphodiesterase from *Penicillium citrinum*

   Trade names : Nuclease E “Amano” 12 (main commercial name)

   Chemical names : Ribonuclease P (EC 3.1.26.5)
3. Names and addresses of basic producers:

   Amano Enzyme Inc.

   2-7, 1-Chome, Nishiki, Naka-ku, Nagoya, Aichi, 460-8630, Japan

   Tel: +81 (0)52-211-3032

   Fax: +81 (0)52-211-3054
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):

   Amano Enzyme Inc.

   2-7, 1-Chome, Nishiki, Naka-ku, Nagoya, Aichi, 460-8630, Japan

   (Attn: Mr Tomonari Ogawa, Director, Quality Assurance Division)

   Tel: +81 (0)52-211-3032

   Fax: +81 (0)52-211-3054

   e-mail: tomonari_ogawa@amano-enzyme.com
6. Justification for use:

The phosphodiesterase is used in the processing of yeast and like products. When applied in that processing, phosphodiesterase increases ribonucleotides by hydrolysing ribonucleic acid (RNA) and improves flavour.

The benefits of using phosphodiesterase is as follows;

As phosphodiesterase hydrolyzes RNA, four kinds of ribonucleotides, guanosine monophosphate (GMP), adenosine monophosphate (AMP), cytidine monophosphate (CMP) and uridine monophosphate (UMP) are formed. Among them, GMP provides the umami taste from the beginning and AMP contributes to umami by conversion to inosine monophosphate (IMP) by deamination. Therefore, it is possible to enhance flavour of yeast and like products.

In the application mentioned above, heating treatment is carried out after the enzyme reaction. Consequently, the phosphodiesterase does not exert any (unintentional) enzymatic activity in the final food. No effect can occur on other food constituents such as carbohydrates, lipids and proteins.

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):

Phosphodiesterase is used as processing aid in the products fallen within the Food Category 12.8 Yeast and like products in accordance with Good Manufacturing Practice (GMP). The dosage of the enzyme varies from 20 to 4000 mg TOS/kg yeast extract.

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))

Currently, this phosphodiesterase preparation is approved in Denmark, France, China, Korea and Japan. In Europe, the dossier is under evaluation.

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

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/Japan) and do not any concerns:

- A 13-week oral toxicity study in rats
- Bacterial reverse mutation test (Ames test)
- Chromosomal aberration test (*in vitro*)

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

The safety of the enzyme is assessed in a battery of toxicology studies investigating its genotoxic and systemic toxicity potential. Daily administration of the enzyme by gavage for 91 continuous days did not result in overt signs of systemic toxicity. Therefore, the highest dose administered, 1867 mg TOS/kg body weight/day, is considered as the NOAEL. A battery of genotoxicity assays was conducted and under the conditions of these assays the enzyme is not a mutagen or a clastogen.

(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)

Phosphodiesterase 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, 10th edition. The details are described below;

1) Produced by non-pathogenic and nontoxigenic strain

2) Produced in accordance with good food manufacturing practice

3) Comply with the purity in the following;

3-1) Lead: Not more than 5 mg/kg

3-2) Microbiological criteria

3-2-1) Salmonella species: absent in 25g of sample

3-2-2) Total coliforms: not more than 30 per gram

3-2-3) Escherichia coli: absent in 25g of sample

3-2-4) Antimicrobial activity: absent in preparation

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

Phosphodiesterase is used in the production of yeast extract, which is used as the key ingredient in seasonings, soups, sauces and gravies.

The enzyme increases the ribonucleotides in the lysate or extract of yeast by hydrolysis of RNA.

The action of the enzyme takes place at the early phase of the process. After that, the enzyme is inactivated during inactivation or sterilization step by high temperature. Therefore, no enzyme activity remains in the final food. Thus the use of the enzyme as processing aid has no influence on the nutritional properties of the final food.

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 dosage of the enzyme varies from 20 to 4000 mg TOS/kg yeast extract and assuming that the maximum dose of yeast extract in food is 2%, the amount of TOS in the final food will be 0.4-80 mg TOS/kg.

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

Based on the conservative calculation by means of the Budget method, assuming that the daily intake of processed foods is 50% of the total solid food intake, i.e. 0.0125 kg/kg bw/day and that the daily intake of soft drinks is 25% of the total beverages intake, i.e. 0.025 l/ kg bw/day, and calculating on basis of the maximal values found in food and beverage, the theoretical total daily intake will be 3.0 mg TOS/kg bw/day.

Other information (as necessary/identified) – None.

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

Immediately

Russian Federation

With this letter, the Russian Federation formally submits comments related to food additives, Nisin (INS 234) and Natamycin (Pimaricin) (INS 235), and would like to include Nisin (INS234) and Natamycin (Pimaricin) (INS 235) to the JECFA priority list. We are requesting safety re-assessment of nisin and natamycin due to emerging data on the bioactivity including the role of nisin and natamycin played in: (i) promoting the antimicrobial resistance as well as speeding up virulence and pathogenic potential of microorganisms which cause food-borne illnesses; (ii) misbalance of immunity status and guts' microflora and other functions in human body. The JECFA re-evaluation is requested to allow CCFA to consider if antibiotics Nisin (INS234) and Natamycin (Pimaricin) (INS 235) should be retained in the GSFA.

1. **Risk assessment of food additives - preservatives Nisin (INS 234) and** Natamycin (Pimaricin) **(INS 235)**

Nisin (INS 234) is a lantibiotic (bacteriocin) antimicrobial peptide produced by *Lactococcus lactis* subsp. Lactis. The CAS Registry Number of nisin is 1414-45-5. The main constituent nisin A has the formula C 143 H 230 N 42 O 37 S 7 and a molecular weight of 3354.11 Daltons.

It is known that nisin A (non-bioengineered nisin) has a relatively narrow range of the antimicrobial activity. Nisin exhibits pore-forming activity and the inhibition of cell membranes biosynthesis of gram-positive microorganisms - *Listeria spp., Staphylococcus spp., Bacillus spp., Clostridium spp*.[1], [2]. At the same time, gram-negative microorganisms (Salmonella spp., Proteus spp., E.coli and another microorganisms of Enterobacteriaceae family, Campylobacter spp.) which are the most important contaminants of ready-to-eat products (as well heat-treated meat and another meat products, milk and milk products, liquid egg products, cheeses, etc.) and most common cause of food poisoning and acute enteric infections are not sensitive to nisin.

Nisin A does not influence the growth of spoiling microorganisms – Proteus spp., Pseudomonas aeruginosa, and a number of species of lactic-acid-producing bacterium, yeasts and moulds. Yeast and moulds are not only resistant to nisin but they are capable of destroying this bactericide.

According to the legislation of the Russian Federation and the Customs Union, the food additive nisin (INS 234) can be used only in a number of food categories: semolina and tapioca puddings and similar products in ML= 3 ppm; ripened cheese and processed cheese in ML=12,5 ppm; curd cheese and unripen cheese type M*ascarpone* in ML=10 ppm; pasteurized liquid egg products in ML= 6,25 ppm.

In 2014-16, the CCFA eWG drafted amendments to the General Standard on Food Additives (Codex Stan 192-1995) proposing that nisin could be used in a number of food categories (including heat-treated processed meat, poultry, and game products, cream (plain), processed cheese, canned or bottled (pasteurized) or retort pouch vegetables etc.) in concentrations 6.25 – 25 ppm.

A number of proposals on expanding the use of nisin in various food categories was discussed for adoption in the eWG in 2016 and adopted by the CCFA.

These proposals are based on the JECFA assessment which found no health risk associated with nisin intake[3]. In our opinion, JECFA assessment of nisin was based primarily on data on chemical safety and did not consider risk assessment of its biological effects.

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[1] Severina E, Severin A, Tomasz A (1998) Antibacterial efficacy of nisin against multidrug-resistant Gram-positive pathogens// J Antimicrob Chemother 41: 341– 347.

[2] FDA (1988) Food and Drug Administration. Nisin preparation: Affirmation of GRAS status as a direct human food ingredient. 11251 ed.

[3] NISIN. First draft prepared by First draft prepared byS. Choudhuri , M. DiNovi1 , P. Sinhaseni and J. Srinivasan /Safety evaluation of certain food additives and contaminants. WHO FOOD ADDITIVES. SERIES:68

In similar fashion, EFSA evaluated nisin and endorsed the ADI for nisin at the level of 0.13 mg/kg bw per day without taking in consideration its antimicrobial activity[4].

It was shown that sub-inhibitory concentrations of nisin induced increased resistance to microorganisms in food (for example *Staphylococcus aureus*). These affects were compared with those of vancomycine. Purified nisin is cytotoxic to several eukaryitic cell types in vitro in concentration of 0,85-3,4 mmol/l. From the reported studies, the order of nisin's cytotoxity is sperm cells>red blood cells>SV40-YC cells> Vero cell lines in concentration of 5-640 ppm[3].

It has been shown that because of its high biological activity lantibiotic nisin can potentially be employed as novel anti-microbial preparation to combat medically significant bacteria and their multi-drug resistant forms[5]. The high effectiveness of nisin was also demonstrated for its use as an antibacterial substance for treatment *Clostridium difficily* and *Listeria monocytogenes* growth control[6],[7]. Several studies reported high activity of nisin S, nisin T and nisin V (novel bioengineered derivatives) against M. tuberculosis (H37Ra), M. kansasii (CIT11/06), M. avium subsp. hominissuis (CIT05/03) and M. avium subsp.paratuberculosis (MAP) (ATCC 19698)[8]. As a result of the pronounced antibacterial activity, nisin-based bacteriocins are regarded as candidates in therapy of infectious diseases caused by microorganisms with multi-AMR[9]. For example, it has been showed that 18 free D-amino acids improve the antibacterial activity of three common antimicrobials, namely nisin, chlorhexidine, and penicillin, against S. mutans and further elevated the effects of these available D-amino acids either alone or in conjunction with nisin on S. mutans biofilms[10]. Notably, nisin A and polymyxin B have been shown to be more effective against Gram negative bacteria when used in combination[10]. Moreover, the high specificity of some bacteriocin (especially obtained by bioengineering methods) into multiple-antibiotic resistant species of microorganisms have made them especially attractive as next-generation antibiotics targeting the multiple-drug resistant pathogens[11].

It had been shown that there are two mechanisms through which bacteria can become resistant to nisin: a shielding mechanism which prevents reaching the target membrane and an enzymatic inactivation mechanism which modifies or destroys the chemical structure of an antibiotic. All data published so far indicate that the former is the main mechanism realized for nisin, also involving several genes. However, it has also been shown that a lactococcal tail-specific protease (NSR) could be responsible for nisin resistance through proteolytic degradation of nisin structure. It was demonstrated that after subculturing in medium contained nisin in concentration 100 ME/ml resistance of S.agalactiae increased 40 times[12].

It is critical to note that nisin was shown to interact with a model cell membrane at a ***micromolar concentration level*** whereas *in vivo* it exerted the bactericidal activity at a nanomolar level, where lipid II is available as a specific docking molecule for binding[13].

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[4] Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on the safety in use of nisin as a food additive in an additional category of liquid eggs and on the safety of nisin produced using a modified production process as a food additive /The EFSA Journal (2006) 314b, 1-8

[5] *Perez R.H., Zendo T., Sonomoto K. Novel bacteriocins from lactic acid bacteria (LAB): various structures and applications //Perez et al. Microbial Cell Factories 2014, 13(Suppl 1):S3*

[6] Gabrielsen C., Brede D.A., Nes I. F., Diep D. B. Circular Bacteriocins: Biosynthesis and Mode of Action// Applied and Environmental Microbiology, Nov. 2014, Vol. 80, N 22 p. 6854–6862

[7] Campion A. et al. In vivo activity of Nisin A and Nisin V against Listeria monocytogenes in mice //BMC Microbiology 2013, P.13:23

[8] James Carrol et. al. Gene encoded antimicrobial peptides, a template for the design of novel anti-mycobacterial drugs //Bioengineered Bugs, November/December 2010, Vol.1:6, P.408-412

[9] Kruszewska D, et al. Mersacidin eradicates methicillin-resistant Staphylococcus aureus (MRSA) in a mouse rhinitis model // J Antimicrob Chemother, 2004, Vol. 54, P. 648–653

[10] Tong Z., Zhang L.,. Ling J. at al. An In Vitro Study on the Effect of Free Amino Acids Alone or in Combination with Nisin on Biofilms as well as on Planktonic Bacteria of Streptococcus mutans// PLOS ONE, June 2014, Volume 9, Issue 6, e99513

[11] Miki Kawada-Matsuo et al. Three Distinct Two-Component Systems Are Involved in Resistance to the Class I Bacteriocins, Nukacin ISK-1 and Nisin A, in Staphylococcus aureus //PLOS ONE, www.plosone.org, 2013, Vol. 8 , Is. 7

[12] Hurst. A. Nisin. / In D. Perlmain and A. 1. Laskin(ed.). Advances in applied microbiology. Academic Press. News York, Vol. 27. 1981, p. 85-123

[13] Sun Z., Zhong J., Liang X. et al. Novel Mechanism for Nisin Resistance via Proteolytic Degradation of Nisin by the Nisin Resistance Protein NSR //ANTIMICROBIAL AGENTS AND C HEMOTHERAPY , May 2009, p. 1964–1973 Vol. 53, No. 5

Mechanisms of resistance to nisin of microorganisms *Staphylococcus aureus,* the most important source of food intoxications[11], and the *Streptococcus bovis* which can cause human cancer[14] are under investigation. Resistance to nisin of such serious pathogens as *Listeria monocitogenes*[15],[16] and *Clostridium botulinum* [17] was recently noted, and mechanisms of *Bacillus subtilis* resistance to nisin are being investigated. These defensive mechanisms are also effective against other lantibiotics such as mersacidin, gallidermin, and subtilin and comprise an important subset of the intrinsic antibiotic resistome of B. subtilis[18].

Nisin could be an inhibitor of lactobacterium growth which is the most important part of normal gut microbiota. For example, nisin was shown to inhibit a growth of *Lactobacillus gasseri* in concentration of 25 ng/ml[19]. This could potentially affect the non-specific immunity status of the population. Antimicrobial peptides play a significant role in building an innate immunity [17],[20].

Nisin's effect on specific bacterial enzymes (α- and β-glucosidases, α-galactosidases and β-glucuronidase) in crop, ileum and caeca was considered in chicken breed with feed supplemented with increasing levels of nisin (100, 300, 900 and 2700 IU nisin/g, respectively). On the 35th day, counts of *Bacteroides* and *Enterobacteriacae* in ileum were significantly (P<0.001) decreased by nisin and salinomycin. Like salinomycin, nisin supplementation improved broiler growth performance in a dose-dependent manner; compared to the nisin group, the body weight gain of the nisin IU=900 and nisin IU=2700 groups was improved by 4,7% and 8,7%, respectively[21].

Nisin may serve as a novel potential therapeutic for treating head and neck squamous cell carcinoma (HNSCC) as it induces preferential apoptosis, cell cycle arrest, and reduces cell proliferation in HNSCC cells, compared with primary keratinocytes in vitro and in vivo conditions[22]. Studies have indicated that the antimicrobial peptide nisin may inhibit the growth of the cariogenic bacteria Streptococcus mutans. Studies showed that D-cysteine (Cys), D- or L-aspartic acid (Asp), and D- or L-glutamic acid (Glu) significantly improve the antibacterial activity of nisin against S. mutans and that the mixture of D-Cys, D-Asp, and D-Glu (3D-AAs) and the mixture of L-Cys, L-Asp, and L-Glu (3L-AAs) at a concentration of 40 mM can prevent S. mutans growth[23].

Naturally novel occurring variant of nisin (Nisin ZP; 95%, high content) induced the greatest level of apoptosis in HNSCC cells. HNSCC cells treated with increasing concentrations of nisin ZP exhibited increasing levels of apoptosis and decreasing levels of cell proliferation, clonogenic capacity, and sphere formation. Nisin ZP induced apoptosis through a calpain-dependent pathway in HNSCC cells but not in human oral keratinocytes. Nisin ZP also induced apoptosis dose-dependently in human umbilical vein endothelial cells (HUVEC) with concomitant decreases in vascular sprout formation in vitro and reduced intratumoral microvessel density in vivo. Nisin ZP reduced tumorigenesis in vivo and long-term treatment with nisin ZP extended survival. In addition, **nisin-treated mice exhibited normal organ histology with no evidence of inflammation, fibrosis or necrosis**. In summary, nisin ZP exhibits greater antitumor effects, and thus has the potential to serve as a novel therapeutic for HNSCC.[24]

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[14] Hilario C. Mantovani and James B. Russell Nisin Resistance of Streptococcus bovis //Applied and Environmental Microbiology, Feb. 2001, p. 808–813

[15] *Teresa M. Bergholz, et al. Nisin Resistance of Listeria monocytogenes Is Increased by Exposure to Salt Stress and Is Mediated via LiaR //Applied and Environmental Microbiology, 2013, Vol. 79 N 18 p. 5682–5688*

[16] *Barry Collins et al. Assessing the Contributions of the LiaS Histidine Kinase to the Innate Resistance of Listeria monocytogenes to Nisin, Cephalosporins, and Disinfectants //Applied and Environmental Microbiology, 2012, Vol. 78, N 8, p. 2923–2929*

[17] *Alejandro S. Mazzota et.al., Nisin Resistance in Clostridium botulinum Spores and Vegetative Cells //Applied and Environmental Microbiology, Feb. 1999, p. 659–664*

[18] *Anthony W. Kingston, Xiaojie Liao, and John D. Helmann Contributions of the σ σ W , σ σ M , and σ σ X Regulons to the Lantibiotic Resistome of Bacillus subtilis //Mol Microbiol., 2013 November, Vol. 90(3), P. 502–518*

[19] *Revilla-Guarinos A., Characterization of a Regulatory Network of Peptide Antibiotic Detoxification Modules in Lactobacillus casei BL23 //Applied and Environmental Microbiology, 2013, Vol.79, N 10, p. 3160–3170*

[20] *Koczulla, A. R., and R. Bals.. Antimicrobial peptides: current status and therapeutic potential. //Drugs, 2003, Vol. 63, P.389–406*

[21] *Damian Józefiak et al. Dietary Nisin Modulates the Gastrointestinal Microbial Ecology and Enhances Growth Performance of the Broiler Chickens//PLOS ONE, www.plosone.org, December 2013, Vol. 8, Is.12*

[22] *Nam E. Joo, et.al Nisin, an apoptogenic bacteriocin and food preservative, attenuates HNSCC tumorigenesis via CHAC1// Cancer Medicine 2012; 1(3): 295–305*

[23] *Zhongchun Tong et al. An In Vitro Study on the Effect of Free Amino Acids Alone or in Combination with Nisin on Biofilms as well as on Planktonic Bacteria of Streptococcus mutans//PLOS ONE.- June 2014.- Vol. 9.- Issue 6.-8 p.- e99513*

[24] *Pachiyappan Kamarajan, et al. Bacteriocin and Food Preservative, Inhibits Head and Neck Cancer Tumorigenesis and Prolongs Survival// PLOS ONE | DOI:10.1371/journal.pone.0131008 July 1, 2015, 20 p.*

It is a well established fact that nisin and other antibiotics have common mechanisms of influence on the genome regulation of a microbial agent. Novel lantibiotics emerge thanks to the upsurge in available bacterial genomes as well as the development of bioinformatics software and search engines designed for the identification of their biosynthetic clusters. In this regard, nisin is at the forefront of bacteriocide-related research.[25]

It was suggested that the salt stress at low temperature could provide cross-protection against nisin and that a potential mechanism of cross-protection is activation of the cell envelope stress response controlled by gene LiaR[26].

At the same time, it was shown that enhanced nisin resistance in some mutants was associated with increased expression of three genes, pbp2229, hpk1021, and lmo2487, encoding a penicillin-binding protein, a histidine kinase, and a protein of an unknown function. The direct role of the three genes just mentioned in nisin resistance was confirmed. The expression of virulence genes in one nisin-resistant mutant and two class IIa bacteriocin-resistant mutants of the same wild-type strain was analyzed and each mutant consistently showed either an increase or a decrease in the expression of virulence genes (prfA-regulated as well as prfA-independent genes). Although the changes were mostly moderate, the consistency indicated that a mutant-specific change in virulence may occur concomitantly with bacteriocin resistance development /Applied and environmental microbiology, Mar. 2004, p. 1669–1679/. Based on the fact that the bacteria of the genus Listeria are ubiquitous and lactobacilli organisms common in the same kinds of food and feed, it is projected that the intensification of resistance genes in Listeria is capable of inducing resistance in lactobacilli resulting synecology these species[27].

It follows that the use of nisin could promote resistance and increase risk of the antibiotic resistance (AMR) transfer to the intestinal microflora as well as speeding up virulence and pathogenic potential of microorganisms which caused food born illnesses.

The high levels of the AMR already seen in the world today are the result of the intensive use and misuse of antibiotics and other antimicrobials in humans, animals (including farmed fish), and crops, as well as the spread of residues of these medicines in soil, crops, and water. Within the broader context of the AMR, resistance to antibiotics is considered the greatest and most urgent global risk requiring international and national attention //www.who.int.

Key facts:

- Antibiotic resistance is one of the biggest threats to global health, food security, and development today.
- Antibiotic resistance can affect anyone, of any age, in any country.
- Antibiotic resistance occurs naturally, but misuse of antibiotics in humans and animals is accelerating the process.
- A growing number of infections – such as pneumonia, tuberculosis, and gonorrhoea – are becoming harder to treat as the antibiotics used to treat them become less effective.
- Antibiotic resistance leads to longer hospital stays, higher medical costs and increased mortality /Antibiotic resistance.-October 2016.-
- Antibiotic resistance causes people to be sick for longer and increases the risk of death. For example, people with MRSA (methicillin-resistant Staphylococcus aureus) are estimated to be 64% more likely to die than people with a non-resistant form of the infection. Resistance also increases the cost of health care with lengthier stays in hospital and more intensive care required. /www.who.int/mediacentre/factsheets

Nisin is used broadly in the veterinary industry (for example as an anti-mastitis product in the form of a wipe-out and an intramammary infusion) and has potential as a clinical antimicrobial[28]. The use of bioengineered bacteriocins for food applications could face consumer resistance as in the case of Genetically Modified Organisms (GMO's)[5]. It is also against widely accepted principle that clinically used medicines should not be used in food industry.

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[25] Des Field et al. Bioengineering of the model lantibiotic nisin// Bioengineered 6:4, 187--192; July/August 2015; © 2015 Taylor & Francis Group, LLC

[26] Bergholz T. M., Tang S., Wiedmann M., Boor K. J. Applied and Environmental Microbiology, September 2013, V. 79, N18, p. 5682–5688

[27] Mazzotta A., Montville T. Listeria monocytogenes resistance to Nisin at 10°C and 30°C// IFT Ann. Meeting'95: Book of Abstracts, 81, D-3,1995

[28] Field D, Begley M., O'Connor P. M. et al. Bioengineered Nisin A Derivatives with Enhanced Activity against Both Gram Positive and Gram Negative Pathogens// PLOS ONE, Oct. 2012, Vol. 7, Is. 10, e46884.

Nisin itself has been subjected to bioengineering for almost twenty years[28]. However only in recent years, researchers greater understanding of lantibiotic biology and the application of bioengineering strategies on a larger-scale, have achieved notable successes with regard to enhancing the antimicrobial activity of lantibiotics against pathogenic bacteria. Both mersacidin and nukacin had been subjected to the comprehensive site-saturation mutagenesis which yielded in the generation of several novel derivatives with enhanced activity compared to the parent peptide against a range of bacterial targets. It is important to note that this improved activity was strain variable, providing further evidence that nisin derivatives can be generated with distinct target specificities.

Thus, it should be noted that bacteriocin's activity of nisin obtained from GMO microorganisms is a lot greater than bacteriocin's activity of nisin A obtained from non-GMO microorganisms, the fact that was not taken into consideration in the JECFA assessment[3]. It becomes a matter of the outmost importance to establish parameters of the safe use and MLs for each type of nisin obtained by biotechnological methods.

This will require conducting risk assessments and establishing specifications for different types of nisin which were obtained by using biotechnological methods.

Recent discussions in the CCFA centered around arguments in favor of nisin based on its technology justification. However, it should be mentioned that the numerous studies on the role that nisin plays in the growth of pathogenic and potentially pathogenic microorganisms require that nisin is treated as a substance of high biological activity. On the other hand, the technological justification based on assumption of inappropriate hygienic conditions should not be accepted by the CCFA as the justification for the use of nisin as a food additive. The storage of food with short shelf life at temperatures above refrigerating should not be encouraged nor accepted.

For examples, in order to ensure safety of products covered by the provisions of Standards for Luncheon Meat (CODEX STAN 89-1981) and for Cooked Cured Chopped Meat (CODEX STAN 98-1981) production, packaging, labeling should be in compliance with HACCP and common hygienic rules.

According to the literature data, nisin is used in canned meat products to reduce the viability of the spores of Clostridium botulinum. Significantly increasing the sensitivity of the bacterial spores to heat, it destroys the cytoplasmic membrane of microbial cells immediately after spore germination. However, evidence of the effectiveness of this method remains questionable.

We conclude that there are evidence-based concerns that allowing the use of nisin (INS 234) across various food categories before the risk assessment is completed represents a substantial risk for the public health. Expanding the use of nisin potentially leads to the development of multi-resistant species of pathogenic and potentially pathogenic microorganisms which are food contaminants, food poisonings and can cause inflammatory illnesses.

Risk estimation of Natamycin (Pimaricin) (INS 235) provided by WHO/JECFA in 2001. Natamycin (pimaricin) is a polyene macrolide antibiotic produced by submerged aerobic fermentation of Streptomyces natalensis and related species. Because natamycin is active against yeasts and moulds, but not bacteria, it is used in foods that undergo a ripening period after processing for the surface treatment of foods. Natamycin is used topically in veterinary medicine to treat mycotic infections, such as ringworm in cattle and horses. Previously, it was used topically against fungal infections of the skin and mucous membranes in humans. Its medical use is now confined to topical treatment of fungal infections of the cornea and the prevention of such infections in contact lens wearers.

At its twentieth meeting, the Committee established an ADI of 0–0.3mg/kg of body weight. The use of natamycin as an antifungal agent in food may result in exposure of the indigenous flora to trace quantities of antimicrobial residues. It was showed possibility antibiotic resistance of Candida albicance into natamycin[29] . These data supported by numerous results of investigation of polyene macrolide antibiotics.

Based on the data on the negative impact of Nisin (INS 234) and Natamycin (Pimaricin) **(INS 235) on human health and the growing use of bioengineered nisin there is an urgent need initiate risk assessment of nisin and** natamycin (pimaricin) **and consider the possibility of exclusion of these antibiotics from the GSFA (Codex STAN 192-1995) and from section 3 of CAC/GL 36-1989.**

[29] *Natamycin (pimaricin).Evaluation of certain food additives and contaminants: fifty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives. Joint FAO/WHO Expert Committee on Food Additives.- WHO technical report series ; 909.-2001 : Rome, Italy.- p.25-29*

Name of Substance(s):	***Nisin INS 234***
Question(s) to be answered by JECFA *(Provide a brief justification of the request in case of re-evaluations)*	***Safety re-assessment and establishment of specifications for different forms of nisin due to emerging data on the bioactivity including nisin's role in promoting the antimicrobial resistance as well as speeding up virulence and pathogenic potential of microorganisms which caused food born illnesses.***

1. Proposal for revision submitted by: **The Russian Federation**
2. Name of substance; trade name(s); chemical name(s): **Nisin**
3. Names and addresses of basicproducers: **N/A**
4. Has the manufacturer made a commitment to provide data?

The Russian Federation will provide data required for re-evaluation

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

The Russian Federation contact point

6. Justification for use: **Used as preservative**
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)

   01.4.3 Clotted cream (plain) 10 mg/kg

   01.6.2 Ripened cheese 12.5 mg/kg

   01.6.5 Cheese analogues 12.5 mg/kg

   01.6.6 Whey protein cheese 12.5 mg/kg

   06.5 Cereal and starch based desserts (e.g., rice pudding, tapioca pudding) 3 mg/kg

   08.2.2 Heat-treated processed meat, poultry, and game products in whole pieces or cuts 25 mg/kg

   08.4 Edible casings (e.g., sausage casings) 7 mg/kg

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 additive is allowed in In the European Union and its members and in the Eurasian Union and its members only in several food categories

9. List of data available (please check, if available) **The Russian Federation is prepared to provide data on the bioactivity of nisin as outline below**

Nutrition Related Studies (Human)

Animal Nutrition Studies

Reviews

1. *Alejandro S. Mazzota et.al., Nisin Resistance in Clostridium botulinum Spores and Vegetative Cells //Applied and Environmental Microbiology, Feb. 1999, p. 659–664*
2. *Anthony W. Kingston, Xiaojie Liao, and John D. Helmann Contributions of the σ σ W , σ σ M , and σ σ X Regulons to the Lantibiotic Resistome of Bacillus subtilis //Mol Microbiol., 2013 November, Vol. 90(3), P. 502–518 sue 6, e99513*
3. *Barry Collins et al. Assessing the Contributions of the LiaS Histidine Kinase to the Innate Resistance of Listeria monocytogenes to Nisin, Cephalosporins, and Disinfectants //Applied and Environmental Microbiology, 2012, Vol. 78, N 8, p. 2923–2929*
4. *Bergholz T. M., Tang S., Wiedmann M., Boor K. J. Applied and Environmental Microbiology, September 2013, V. 79, N18, p. 5682–5688*
5. *Campion A. et al. In vivo activity of Nisin A and Nisin V against Listeria monocytogenes in mice //BMC Microbiology 2013, P.13:23*
6. *Damian Józefiak et al. Dietary Nisin Modulates the Gastrointestinal Microbial Ecology and Enhances Growth Performance of the Broiler Chickens//PLOS ONE, www.plosone.org, December 2013, Vol. 8, Is.12*
7. *Des Field et al. Bioengineering of the model lantibiotic nisin// Bioengineered 6:4, 187--192; July/August 2015; © 2015 Taylor & Francis Group, LLC*

8. FDA (1988) Food and Drug Administration. Nisin preparation: Affirmation of GRAS status as a direct human food ingredient. 11251 ed.
9. *Field D, Begley M., O'Connor P. M. et al. Bioengineered Nisin A Derivatives with Enhanced Activity against Both Gram Positive and Gram Negative Pathogens// PLOS ONE, Oct. 2012, Vol. 7, Is. 10, e46884.*
10. *Gabrielsen C., Brede D.A., Nes I. F., Diep D. B. Circular Bacteriocins: Biosynthesis and Mode of Action// Applied and Environmental Microbiology, Nov. 2014, Vol. 80, N 22 p. 6854–6862*
11. *Hilario C. Mantovani and James B. Russell Nisin Resistance of Streptococcus bovis //Applied and Environmental Microbiology, Feb. 2001, p. 808–813*
12. *Hurst. A. Nisin. / In D. Perlmain and A. 1. Laskin(ed.). Advances in applied microbiology. Academic Press. News York, Vol. 27. 1981, p. 85-123*
13. *James Carrol et. al. Gene encoded antimicrobial peptides, a template for the design of novel anti-mycobacterial drugs //Bioengineered Bugs, November/December 2010, Vol.1:6, P.408-412*
14. *Koczulla, A. R., and R. Bals.. Antimicrobial peptides: current status and therapeutic potential. //Drugs, 2003, Vol. 63, P.389–406*
15. *Kruszewska D, et al. Mersacidin eradicates methicillin-resistant Staphylococcus aureus (MRSA) in a mouse rhinitis model // J Antimicrob Chemother, 2004, Vol. 54, P. 648–653*
16. *Mazzotta A., Montville T. Listeria monocytogenes resistance to Nisin at 10°C and 30°C// IFT Ann. Meeting'95: Book of Abstracts, 81, D-3,1995*
17. *Miki Kawada-Matsuo et al. Three Distinct Two-Component Systems Are Involved in Resistance to the Class I Bacteriocins, Nukacin ISK-1 and Nisin A, in Staphylococcus aureus //PLOS ONE, www.plosone.org, 2013, Vol. 8 , Is. 7*
18. *Nam E. Joo, et.al Nisin, an apoptogenic bacteriocin and food preservative, attenuates HNSCC tumorigenesis via CHAC1// Cancer Medicine 2012; 1(3): 295–305*
19. NISIN. First draft prepared by First draft prepared byS. Choudhuri , M. DiNovi1 , P. Sinhaseni and J. Srinivasan /Safety evaluation of certain food additives and contaminants. WHO FOOD ADDITIVES. SERIES:68
20. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on the safety in use of nisin as a food additive in an additional category of liquid eggs and on the safety of nisin produced using a modified production process as a food additive /The EFSA Journal (2006) 314b, 1-8
21. *Pachiyappan Kamarajan, et al. Bacteriocin and Food Preservative, Inhibits Head and Neck Cancer Tumorigenesis and Prolongs Survival// PLOS ONE | DOI:10.1371/journal.pone.0131008 July 1, 2015, 20 p.*
22. *Perez R.H., Zendo T., Sonomoto K. Novel bacteriocins from lactic acid bacteria (LAB): various structures and applications //Perez et al. Microbial Cell Factories 2014, 13(Suppl 1):S3*
23. *Revilla-Guarinos A., Characterization of a Regulatory Network of Peptide Antibiotic Detoxification Modules in Lactobacillus casei BL23 //Applied and Environmental Microbiology, 2013, Vol.79, N 10, p. 3160–3170*
24. Severina E, Severin A, Tomasz A (1998) Antibacterial efficacy of nisin against multidrug-resistant Gram-positive pathogens// J Antimicrob Chemother 41: 341– 347.
25. *Sun Z., Zhong J., Liang X. et al. Novel Mechanism for Nisin Resistance via Proteolytic Degradation of Nisin by the Nisin Resistance Protein NSR //ANTIMICROBIAL AGENTS AND C HEMOTHERAPY , May 2009, p. 1964–1973 Vol. 53, No. 5*
26. *Teresa M. Bergholz, et al. Nisin Resistance of Listeria monocytogenes Is Increased by Exposure to Salt Stress and Is Mediated via LiaR //Applied and Environmental Microbiology, 2013, Vol. 79 N 18 p. 5682–5688*
27. *Tong Z., Zhang L.,. Ling J. at al. An In Vitro Study on the Effect of Free Amino Acids Alone or in Combination with Nisin on Biofilms as well as on Planktonic Bacteria of Streptococcus mutans// PLOS ONE, June 2014, Volume 9, Is*
28. *Zhongchun Tong et al. An In Vitro Study on the Effect of Free Amino Acids Alone or in Combination with Nisin on Biofilms as well as on Planktonic Bacteria of Streptococcus mutans//PLOS ONE.- June 2014.- Vol. 9.- Issue 6.-8 p.- e99513*
29. *//www.who.int.*

Other information (as necessary/identified)

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

The Russian Federation is ready to provide the data in May 2017

Name of Substance(s):	***Pimaricin, Natamycin INS 235***
Question(s) to be answered by JECFA *(Provide a brief justification of the request in case of re-evaluations)*	***Safety re-assessment and establishment of specifications for different forms of nisin due to emerging data on the bioactivity including nisin's role in promoting the antimicrobial resistance as well as speeding up virulence and pathogenic potential of microorganisms which caused food born illnesses.***

1. Proposal for revision submitted by: **The Russian Federation**
2. Name of substance; trade name(s); chemical name(s): **pimaricin, natamycin**
3. Names and addresses of basic producers: **N/A**
4. Has the manufacturer made a commitment to provide data?

The Russian Federation will provide data required for re-evaluation

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

The Russian Federation contact point

6. Justification for use: **Used as preservative**
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)

   01.6.1 Unripened cheese 40 mg/kg

   01.6.2 Ripened cheese 40 mg/kg

   01.6.4 Processed cheese 40 mg/kg 2006 3 & 80

   01.6.5 Cheese analogues 40 mg/kg 2006 3 & 80

   01.6.6 Whey protein cheese 40 mg/kg 2006 3 & 80

   08.2.1.2 Cured (including salted) and dried non-heat treated processed meat, poultry, and game products in whole pieces or cuts 6 mg/kg

   08.3.1.2 Cured (including salted) and dried non-heat treated processed comminuted meat, poultry, and game products 20 mg/kg

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 additive is allowed in In the European Union and its members and in the Eurasian Union and its members only in several food categories

9. List of data available (please check, if available) **The Russian Federation is prepared to provide data on the bioactivity of nisin as outline below**

Nutrition Related Studies (Human)

Animal Nutrition Studies

Reviews

Natamycin (pimaricin). Evaluation of certain food additives and contaminants: fifty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives. Joint FAO/WHO Expert Committee on Food Additives.- WHO technical report series ; 909.-2001 : Rome, Italy.- p.25-29

//www.who.int.

Other information (as necessary/identified)

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

The Russian Federation is ready to provide the data in May 2017