CODEX ALIMENTARIUS COMMISSION

Food and Agriculture Organization of the United Nations

World Health Organization

E

Viale delle Terme di Caracalla, 00153 Rome, Italy - Tel: (+39) 06 57051 - E-mail: codex@fao.org - www.codexalimentarius.org

**Agenda Item 5e** **CX/FA 16/48/11**

February 2016

**JOINT FAO/WHO FOOD STANDARDS PROGRAMME**

**CODEX COMMITTEE ON FOOD ADDITIVES**

**Forty-eighth Session**

**Xi'an, China, 14-18 March 2016**

# PROPOSALS FOR NEW AND/OR REVISION OF FOOD ADDITIVE PROVISIONS

**Comments (replies to CL 2015/12-FA) of Japan, Russian Federation, CEFIC, IADSA and ISDI**

## JAPAN

| | |
|---|---|
| **THE PROPOSAL IS SUBMITTED BY:** | Japan |
| **IDENTITY OF THE FOOD ADDITIVE:** | |
| **Name of the Additive**<br>*As listed in Class Names and the International Numbering System (INS) - CAC/GL 36-1989* | *Advantame* |
| **INS Number** | *969* |
| **Functional Class**<br>*As listed in Class Names and the International Numbering System (INS) - CAC/GL 36-1989* | *Sweetener*<br>*Flavour enhancer* |

**PROPOSED USE(S) OF THE FOOD ADDITIVE:** The proposal for ■ a new provision; or ☐ revising an existing provision

| Food Category No. | Food Category Name | Maximum Use Level ([1]) | Comments |
|---|---|---|---|
| 01.1.2 | Dairy-based drinks, flavoured and/or fermented (e.g. chocolate milk, cocoa, eggnog, drinking yoghurt, whey-based drinks) | 6 mg/kg | Besides adding sweetness, advantame can enhance flavor of milk-added products of this food category. |
| 01.3.2 | Beverage whiteners | 60 mg/kg | |
| 01.4.4 | Cream analogues | 10 mg/kg | |
| 01.5.2 | Milk and cream powder analogues | 20 mg/kg | |
| 01.6.1 | Unripened cheese | 10 mg/kg | |
| 01.6.5 | Cheese analogues | 10 mg/kg | |
| 01.7 | Dairy-based desserts (e.g., pudding, fruit or flavoured yoghurt) | 10 mg/kg | |
| 02.3 | Fat emulsions mainly of type oil-in-water, including mixed and/or flavoured products based on fat emulsions | 10 mg/kg | Advantame can be used in this category because it is highly soluble in fat in comparison with other sweeteners. |
| 02.4 | Fat-based desserts excluding dairy-based dessert products of food category 01.7 | 10 mg/kg | |
| 03 | Edible ices, including sherbet and sorbet | 10 mg/kg | Besides adding sweetness, advantame can enhance flavor of fruit and/or milk-added products of this food category. |
| 04.1.2.1 | Frozen fruit | 20 mg/kg | |

| | | | |
|---|---|---|---|
| 04.1.2.2 | Dried fruit | 20 mg/kg | Besides adding sweetness, advantame can enhance flavor of fruit of this food category. |
| 04.1.2.3 | Fruit in vinegar, oil, or brine | 3 mg/kg | |
| 04.1.2.4 | Canned or bottled (pasteurized) fruit | 10 mg/kg | |
| 04.1.2.5 | Jams, jellies, marmelades | 10 mg/kg | |
| 04.1.2.6 | Fruit-based spreads (e.g., chutney) excluding products of food category 04.1.2.5 | 10 mg/kg | |
| 04.1.2.7 | Candied fruit | 20 mg/kg | |
| 04.1.2.8 | Fruit preparations, including pulp, purees, fruit toppings and coconut milk | 10 mg/kg | |
| 04.1.2.9 | Fruit-based desserts, including fruit-flavoured water-based desserts | 10 mg/kg | |
| 04.1.2.10 | Fermented fruit products | 10 mg/kg | |
| 04.1.2.11 | Fruit fillings for pastries | 10 mg/kg | |
| 04.1.2.12 | Cooked fruit | 10 mg/kg | |
| 04.2.2.1 | Frozen vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweeds, and nuts and seeds | 10 mg/kg | |
| 04.2.2.2 | Dried vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweeds, and nuts and seeds | 10 mg/kg | |
| 04.2.2.3 | Vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), and seaweeds in vinegar, oil, brine, or soybean sauce | 3 mg/kg | Note 144: For use in sweet and sour products only.<br>Besides adding sweetness, advantame can enhance flavor of fruit of this food category. |
| 04.2.2.4 | Canned or bottled (pasteurized) or retort pouch vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), and seaweeds | 10 mg/kg | Besides adding sweetness, Advantame can enhance flavor of fruit of this food category. |
| 04.2.2.5 | Vegetable (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweed, and nut and seed purees and spreads (e.g., peanut butter) | 10 mg/kg | |
| 04.2.2.6 | Vegetable (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweed, and nut and seed pulps and preparations (e.g., vegetable desserts and sauces, candied vegetables) other than food category 04.2.2.5 | 10 mg/kg | |
| 04.2.2.7 | Fermented vegetable (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera) and seaweed products, excluding fermented soybean products of food categories 06.8.6, 06.8.7, 12.9.1, 12.9.2.1 and 12.9.2.3 | 25 mg/kg | |
| 04.2.2.8 | Cooked or fried vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), and seaweeds | 10 mg/kg | |
| 05.1.1 | Cocoa mixes (powders) and cocoa mass/cake | 30 mg/kg | Note 97: On the final cocoa and chocolate product basis. |
| 05.1.2 | Cocoa mixes (syrups) | 10 mg/kg | |
| 05.1.3 | Cocoa-based spreads, including fillings | 30 mg/kg | |
| 05.1.4 | Cocoa and chocolate products | 30 mg/kg | |

| | | | |
|---|---|---|---|
| 05.1.5 | Imitation chocolate, chocolate substitute products | 30 mg/kg | |
| 05.2.1 | Hard candy | 30 mg/kg | New note: Except for use in microsweets and breath freshening mints at 100 mg/kg. |
| 05.2.2 | Soft candy | 30 mg/kg | New note: Except for use in microsweets and breath freshening mints at 100 mg/kg. |
| 05.2.3 | Nougats and marzipans | 30 mg/kg | |
| 05.3 | Chewing gum | 100 mg/kg | |
| 05.4 | Decorations (e.g., for fine bakery wares), toppings (non-fruit) and sweet sauces | 10 mg/kg | |
| 06.3 | Breakfast cereals, including rolled oats | 10 mg/kg | |
| 06.5 | Cereal and starch based desserts (e.g., rice pudding, tapioca pudding) | 10 mg/kg | |
| 07.1.5 | Steamed breads and buns | 10 mg/kg | |
| 07.2 | Fine bakery wares (sweet, salty, savoury) and mixes | 17 mg/kg | Note 165: For use in products for special nutritional use only. |
| 09.2 | Processed fish and fish products, including mollusks, crustaceans, and echinoderms | 3 mg/kg | Note 144: For use in sweet and sour products only. |
| 09.3 | Semi-preserved fish and fish products, including mollusks, crustaceans, and echinoderms | 3 mg/kg | Note 144: For use in sweet and sour products only. |
| 09.4 | Fully preserved, including canned or fermented fish and fish products, including mollusks, crustaceans, and echinoderms | 3 mg/kg | Note 144: For use in sweet and sour products only. |
| 10.4 | Egg-based desserts (e.g., custard) | 10 mg/kg | |
| 11.4 | Other sugars and syrups (e.g., xylose, maple syrup, sugar toppings) | 30 mg/kg | Note 159: For use in pancake syrup and maple syrup only. |
| 11.6 | Table-top sweeteners, including those containing high-intensity sweeteners | GMP | |
| 12.2.2 | Seasonings and condiments | 20 mg/kg | |
| 12.3 | Vinegars | 30 mg/kg | |
| 12.4 | Mustards | 3.5 mg/kg | |
| 12.5 | Soups and broths | 12 mg/kg | Note XS117: Excluding products conforming to the Codex Standard for Bouillons and Consommés (CODEX STAN 117-1981). |
| 12.6 | Sauces and like products | 3.5 mg/kg | |
| 12.7 | Salads (e.g., macaroni salad, potato salad) and sandwich spreads excluding cocoa- and nut-based spreads of food categories 04.2.2.5 and 05.1.3 | 3.5 mg/kg | Note: 166 For use in milk-based sandwich spreads only. |
| 13.3 | Dietetic foods intended for special medical purposes (excluding products of food category 13.1) | 10 mg/kg | |
| 13.4 | Dietetic formulae for slimming purposes and weight reduction | 8 mg/kg | |
| 13.5 | Dietetic foods (e.g., supplementary foods for dietary use) excluding products of food categories 13.1 - 13.4 and 13.6 | 10 mg/kg | |

| | | | |
|---|---|---|---|
| 13.6 | Food supplements | 55 mg/kg | |
| 14.1.3.1 | Fruit nectar | 6 mg/kg | |
| 14.1.3.2 | Vegetable nectar | 6 mg/kg | |
| 14.1.3.3 | Concentrates for fruit nectar | 6 mg/kg | Note 127: On the served to the consumer basis. |
| 14.1.3.4 | Concentrates for vegetable nectar | 6 mg/kg | Note 127: On the served to the consumer basis. |
| 14.1.4 | Water-based flavoured drinks, including "sport," "energy," or "electrolyte" drinks and particulated drinks | 6 mg/kg | |
| 14.1.5 | Coffee, coffee substitutes, tea, herbal infusions, and other hot cereal and grain beverages, excluding cocoa | 6 mg/kg | Note 160: For use in ready-to-drink products and pre-mixes for ready-to-drink products only. |
| 14.2.7 | Aromatized alcoholic beverages (e.g., beer, wine and spirituous cooler-type beverages, low alcoholic refreshers) | 6 mg/kg | |
| 15.0 | Ready-to-eat savouries | 5 mg/kg | |
| | | | |

**EVALUATION BY JECFA**

| | |
|---|---|
| **Evaluation by JECFA**<br>*Reference to the JECFA evaluation (including year and JECFA session of evaluation; full ADI (numerical or "not specified"); specifications monograph).* | Safety evaluation was conducted in the seventy-seventh meeting of the Joint FAO/WHO Expert Committee on Food Additives. An ADI of 0-5 mg/kg bw was set.<br>Specification monograph was published in the 80th JECFA meeting report. |

**JUSTIFICATION**

| | |
|---|---|
| **Justification for use and technological need**<br>*Supporting information based on the criteria in Section 3.2 of the Preamble of the General Standard for Food Additives (i.e. has an advantage, does not present an appreciable health risk, serves a technological function).* | The intended use of Advantame is to replace caloric sugars (sucrose, glucose, fructose, etc.) in the categories of foods in which high-intensity sweeteners are permitted by the GSFA.<br>The actual use levels that will be incorporated to calorie-reduced foods will vary between individual manufacturers and products, but will be covered by current Good Manufacturing Practices (GMP) and/or the maximum permitted usage levels which will be derived on a sweetness basis of aspartame within the individual food categories.<br>Furthermore advantame is a flavor enhancer, enhancing many flavors such as dairy, fruit, citrus, mint, etc. and can be used to extend chew time in chewing gum. Advantame is also a tool in masking off-tastes of functional ingredients, such as added proteins, vitamins, minerals, etc.<br>In addition, advantame can be used as a sweetener for Fat-based products, fat emulsions and other high fat content products because it is highly soluble in fat in comparison with other sweeteners. |
| **Safe use of additive: Dietary intake assessment** (*as appropriate*) | *Table 3 additive:*<br>☐ *Yes*<br>■ *No (Please provide information on dietary intake assessment below)*<br>FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS STATISTICS DIVISION (FAOSTAT) database includes estimates for all kind of sweetener supply for all countries and regions (WHO, 2011). Assuming, as a worst case scenario, that Advantame would replace all sugar supply, intake values of Advantame can be estimated; however, such intake estimates would be highly conservative since it is unlikely that Advantame would entirely replace sugar supply and the consumption of sweeteners should be smaller than the sweeteners supply. Considering that Advantame is approximately 20,000 times as sweet as sucrose, per capita intake of Advantame of up to 8.15 mg/day (0.136 mg/kg body weight/day) is estimated based on commodity sweetener. |

<table>
<tr><td></td><td>
<table>
<tr><td></td><td>Commodity Sweetener (g/capita/day)</td><td colspan="2">Predicted intakes of Advantame (mg/kg body weight)</td></tr>
<tr><td></td><td></td><td>mg/capita/day</td><td>mg/kg BW/day</td></tr>
<tr><td>Africa</td><td>45</td><td>2.25</td><td>0.038</td></tr>
<tr><td>Northern America</td><td>161</td><td>8.05</td><td>0.134</td></tr>
<tr><td>Southern America</td><td>107</td><td>5.35</td><td>0.089</td></tr>
<tr><td>Asia</td><td>47</td><td>2.35</td><td>0.039</td></tr>
<tr><td>Europe</td><td>111</td><td>5.55</td><td>0.093</td></tr>
<tr><td>Australia & New Zealand</td><td>130</td><td>6.50</td><td>0.108</td></tr>
</table>
Commodity Sweetener is the amount of monosaccharides (glucose and fructose) and disaccharides (sucrose and saccharose) used for sweetening.<br><br>Proposed maximum use levels of advantame are calculated from those of aspartame within corresponding food categories by dividing 100, as the sweetness of advantame is considered to be 100 times higher than that of aspartame. JECFA has established an ADI of 0 – 5 mg/kg bw for advantame. Considering the above, even if advantame would replace all use of Commodity Sweeteners, intake of advantame does not exceed upper bound of the ADI of advantame, and the use of advantame is of negligible safety concern.</td></tr>
<tr><td>Justification that the use does not mislead consumer</td><td>An increase of numbers of sweeteners permitted by the GSFA would be advantageous for the consumers who are forced to eat Sugar-Free, Low-Calorie or Sugarless foods only due to their physical problems.<br><br>By properly displayed on the label in accordance with the law in each regions, consumers easily understand that advantame is contained in foods and the product has a feature that is different from the products that were made only with sugar.</td></tr>
</table>

**([1])** Proposed maximum use levels of advantame are derived on a sweetness basis of aspartame within the corresponding food categories.

## RUSSIAN FEDERATION

Russian Federation considered it is necessary to fulfill safety reevaluation of food additive Nisin (INS 234) (see attached form (Annex 1) and below information).

**NISIN (INS 234)**

According to the evidence of scientific data nisin (INS 234) is lantibiotic (bacteriocin) antimicrobial peptide produced by *Lactococcus lactis* subsp. Lactis. (Lantibiotics are a class of peptide antibiotics (bacteriocine) that contain the characteristic polycyclic amino acids lanthionine or methyllanthionine, as well as the unsaturated amino acids dehydroalanine and 2-aminoisobutyric acid). 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 specter of antimicrobial activity. Nisin exhibits pore-forming activity and the inhibition of cell's membranes biosynthesis of gram-positive microorganisms - *Listeria* spp., *Staphylococcus* spp., *Bacillus* spp., *Clostridium* spp.[1], [2] Simultaneously, gram-negative microorganisms which are the most important contaminants of ready-to-eat heat-treated meat products (Salmonella spp., Proteus spp., E.coli and another microorganisms of Enterobacteriaceae family, Campylobacter spp.) and caused most cases of food poisoning and acute enteric infections not sensitive to nisin.

[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.

**Nisin A does not influence the growth of spoiling microorganisms – *Proteus* spp., *Pseudomonas aeruginosa*, and a lot of number species of lactic-acid-producing bacterium, yeasts and moulds. Yeast and moulds are not only resistant to nisin, but also could quickly destroy this bacteriocin.**

According to the legislation of the Russian Federation and Customs Union the food additive nisin (INS 234) can be used only in a numbered 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 unripened cheese type M*ascarpone* in ML=10 ppm; pasteurized liquid egg products in ML= 6,25 ppm.

However according to proposals of CCFA elaborated by eWG , prepared in 2014 and in 2015 years, on amendments to the General Standard on Food Additives (Codex STAN 192-1995) nisin could be used in a number of groups of food products, including heat-treated processed meat, poultry, and game products in more higher concentration ML= 25 ppm.

These proposals are based on the conclusion of JECFA about safety of nisin[3]. However, in our opinion, JECFA have taken in consideration (as a base) the safety of nisin as chemical substance, but did not take into account possibility of its negative biological effects.

The EFSA evaluated nisin and endorsed the ADI of 0.13 mg nisin/kg bw per day without taking in consideration its antimicrobial activity[4].

It was showed that sub-inhibitory concentrations of nisin induced increased resistance of microorganisms in food (for example *Staphylococcus aureus*) to nisin. These affects were compared with those of vancomycine. Purified nisin is cytotoxic to a number of 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's cells>red blood cells>SV40-YC cells> Vero cell lines in concentration of 5-640 ppm[3].

As a result of high biological activities, it has been showed that lantibiotic nisin can potentially be employed as novel anti-microbials preparation to combat medically significant bacteria and their multi-drug resistant forms[5]. The higher effectiveness of nisin was showed in case of using as antibacterial substance for treatment in purpose *of Clostridium difficily* and *Listeria monocytogenes* growth control[6],[7]. Successful investigations showed higher 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 higher antibacterial activity, bacteriocins are candidates for using in therapy of infection diseases caused by microorganisms with multiresistance into antibiotics[9] For example it had 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 evaluated 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 than when either is used alone[11]. Moreover the higher specificity of some bacteriocin (especially obtained by bioengineering's methods) into multiple-antibiotic resistant species of microorganisms have made them especially attractive as next-generation antibiotics targeting the multiple-drug resistant pathogens[12]. It is possible because the action of nisin are similar to antibiotics.

It had been showed that there are overall two mechanisms by which bacteria can become resistant to nisin: a shielding mechanism to prevent it from reaching the target membrane and an enzymatic inactivation mechanism by modification or destruction of the antibiotics (3). All data published so far indicate that the former is the main mechanism, with the involvement of a number of genes. However, had been showed that a lactococcal tail-specific protease (NSR) responsible for nisin resistance through proteolytic degradation of nisin.

---

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

[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] Tong Z., Zhang Y., Ling J. et al. In Vitro Study on the Effects of Nisin on the Antibacterial Activities of 18 Antibiotics against Enterococcus faecalis// PLOS ONE, Feb. 2014, Vol., 9, Is, 2, e89209

[12] 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

It was showed that after one procedure of subculturing in medium contained nisin in concentration 100 ME/ml resistanse of S.agalactiae incesed in 40 times[13].

It has been reported that nisin acts on a model membrane at a ***micromolar concentration level,*** whereas it exerts bactericidal activity at a nanomolar level in vivo, where lipid II is available as a specific docking molecule for binding[14].

Mechanisms of resistance to nisin of microorganisms *Staphylococcus aureus* - the most important source of food intoxications[15], and the *Streptococcus bovis* which can cause human cancer[16] are under investigation. Resistance to nisin of such serious pathogens as *Listeria monocitogenes*[17,18] and *Clostridium botulinum* [19] was noted. Mechanisms of *Bacillus subtilis* resistance to nisin is 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[20].

It is showed that nisin could be inhibitor of lactobacterium growth which is the most important part of normal gut microbiota. For example, nisin could inhibit a growth of *Lactobacillus gasseri* in concentration of 25 ng/ml[21]. As a result this process can inhibited the non-specific immunity status of population. Antimicrobial peptides play a significant role in building an innate immunity [17,22].

The possibility of nisin influence on the activity of specific bacterial enzymes (α- and β-glucosidases, α-galactosidases and β-glucuronidase) in crop, ileum and caeca was taken for the justification of its use in case of chicken breed. The nisin diets supplemented with increasing levels (100, 300, 900 and 2700 IU nisin/g, respectively). At the 35-th 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[23].

**It is a verified fact that nisin and another antibiotics have common mechanisms of influence on microbial agent's genome regulation.**

It was suggested that 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[24].

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 unknown function, respectively. The direct role of the three genes in nisin resistance was determined. 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 mostly were moderate, the consistency indicates 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

[13] 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

[14] 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

[15] 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

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

[17] 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

[18] 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

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

[20] 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

[21] 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

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

[23] 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

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

of resistance genes in Listeria is capable of inducing resistance in lactobacilli resulting synecology these species[25].

**Therefore using of nisin can promote resistance and increase the risk of transfer of antibiotic resistance to representatives of the intestinal microflora, as well as speeding up virulence and pathogenic potential of microorganisms which cause food borne illnesses.**

Nisin is also used in the veterinary industry (for example as an anti-mastitis product in the form of Wipe OutH, and an intramammary infusion) and has potential as a clinical antimicrobial[26]. The use of bioengineered bacteriocins for food applications could face consumer resistance as in the case Genetically Modified Organisms (GMO's)[27]. However according to established principles medicaments used in clinic cannot be used in food industry.

Nisin itself has been subjected to bioengineering for almost twenty years[28]. However only in recent years researchers better 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 have been the subject of comprehensive site-saturation mutagenesis approaches which have resulted 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 much higher than bacteriocin's activity of nisin A obtained from non GMO microorganisms which was not taken into consideration by JECFA[3]. In this way it is necessary to establish safety records and ML for each type of nisin obtained by using biotechnological methods.

**Therefore risk assessments and specifications of different types of nisin which were obtained by using biotechnological methods should be provided.**

In proposed by CCFA data there are only arguments in favor of nisin based on its technology justification. However it should be mentioned that the higher level of nisin influence at the growth of pathogenic and potentially pathogenic microorganisms define it as substance with higher level of biological activity. For another thing, violation of hygienic regulations cannot be the justification of the food additive use. The storage of food with short life-cycle without refrigerators is impossible.

**To provide safety of the products covered (for example) by the provisions of Standards for Luncheon Meat (CODEX STAN 89-1981) and for Cooked Cured Chopped Meat (CODEX STAN 98-1981) it is enough if its production, packaging, labeling will be in compliance with demands of GMP and common hygienic rules.**

According to scientific literature the use of nisin in canned meat products to reduce the viability of the spores of Clostridium botulinum (as processing aids). 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 is incomplete.

According to it, we suppose it is not reasonable to expend nisin (INS 234) using into new food categories before its risk estimation and risk assessment is implemented. Expanding of nisin using can lead to the development of multiresistant species of pathogenic and potentially pathogenic microorganisms which are food contaminants, food poisonings and can cause inflammatory disease.

**Based on the data on the possible negative impact nisin on the human health and the fact of bioengineered nisin use it is necessary to conduct the nisin risk revision (**and may be consider the possibility of exclusion of this food additive from the relevant INS list of CODEX STAN 192-1995**).**

[25] 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

[26] 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.

[27] Perez et al . Novel bacteriocins from lactic acid bacteria (LAB): various structures and applications// Microbial Cell Factories, 2014, Vol.13 (Suppl 1):S3

[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, Issue 10, e46884

**Annex 1**

| | | | |
|---|---|---|---|
| **THE PROPOSAL IS SUBMITTED BY:** | | Russian Federation | |
| **IDENTITY OF THE FOOD ADDITIVE:** | | | |
| **Name of the Additive**<br>*As listed in Class Names and the International Numbering System (INS) - CAC/GL 36-1989* | | **NISIN.**<br>The CAS Registry Number of nisin is 1414-45-5. | |
| **INS Number** | | **INS 234** | |
| **Functional Class**<br>*As listed in Class Names and the International Numbering System (INS) - CAC/GL 36-1989* | | Preservative | |
| **PROPOSED USE(S) OF THE FOOD ADDITIVE ([1]):** | | The proposal for revising an existing provision of safety evaluation prepared in 2013 (WHO food additives series ; 68) | |
| **Food Category No. ([2])** | **Food Category Name ([2])** | **Maximum Use Level ([3])** | **Comments ([4])** |
| **EVALUATION BY JECFA:** | | | |
| **Evaluation by JECFA**<br>*Reference to the JECFA evaluation (including year and JECFA session of evaluation; full ADI (numerical or "not specified"); specifications monograph).* | | NISIN. First draft prepared by First draft prepared by S. Choudhuri, M. DiNovi1, P. Sinhaseni and J. Srinivasan /Safety evaluation of certain food additives and contaminants. WHO FOOD ADDITIVES. SERIES: 68<br>ADI - 0–2 mg/kg bw | |

**JUSTIFICATION:**

1. Nisin A does not influence the growth of spoiling microorganisms – *Proteus* spp., *Pseudomonas aeruginosa*, and a lot of species of lactic-acid-producing bacterium, yeasts and moulds. Yeast and moulds are not only resistant to nisin, but also can quickly destroy this bacteriocin.

2. It is a verified fact that nisin and another antibiotics have common mechanisms of influence on microbial agent's genome regulation. Therefore using of nisin can promote resistance and increase risk the transfer of antibiotic resistance to representatives of the intestinal microflora, as well as speeding up virulence and pathogenic potential of microorganisms which cause food borne illnesses.

3. Risk assessments and specifications of different types of nisin which were obtained by using biotechnological methods should be provided.

4. Based on the data on the possible negative impact nisin on the human health and the fact of bioengineered nisin use it is necessary to conduct the nisin risk revision

(Additional information provided in letter).

## CONSEIL EUROPEEN DE L'INDUSTRIE CHIMIQUE (CEFIC)

| | | |
|---|---|---|
| **THE PROPOSAL IS SUBMITTED BY:** | *Cefic (European Chemical Industry Council)* | |
| **IDENTITY OF THE FOOD ADDITIVE:** | | |
| **Name of the Additive**<br>*As listed in Class Names and the International Numbering System (INS) - CAC/GL 36-1989* | *Magnesium Stearate* | |
| **INS Number** | *INS 470(iii)* | |
| **Functional Class**<br>*As listed in Class Names and the International Numbering System (INS) - CAC/GL 36-1989* | *Functional class:* | *Technological purpose* |
| | *Anticaking agent* | *Anticaking agent, lubricant, release agent* |
| | *Emulsifier* | *Emulsifier* |
| | *Thickener* | *Thickener, binder* |

| **PROPOSED USE(S) OF THE FOOD ADDITIVE ([1]):** | | The proposal for X a new provision; | |
|---|---|---|---|
| **Food Category No. ([2])** | **Food Category Name ([2])** | **Maximum Use Level ([3])** | **Comments ([4])** |
| 05.2 | Confectionery including hard and soft candy, nougats, etc. other than food categories 05.1, 05.3 and 05.4 | *13,000 mg/kg* | *Lubricant / release agent / binder in hard candy, pressed mint and mint pastille* |
| 05.3 | Chewing gum | *20,000 mg/kg* | *Surface active agent, intended as a "flow improvement agent"*<br>*Drying agent* |
| 13.6 | Food Supplements | *30,000 mg/kg* | *Lubricant / release agent in chewable tablets, capsules, powders* |
| 07.0 | Bakery wares | *2,500 mg/kg* | *Emulsifier / Binder in rusks, baking powder* |
| 12.2.1 | Herbs and Spices | *10,000 mg/kg* | *Anticaking agent in hydrophobic powdered spices and herbs* |
| Table 3 | May be used in Table 3 foods under the conditions of good manufacturing practices (GMP) as outlined in the Preamble of the Codex GSFA. | *GMP* | *Lubricant / release agent / binder / Emulsifier / Anticaking agent*<br>*INS 470 (i) and INS 470 (ii) are both listed in Table 3* |

**EVALUATION BY JECFA:**

| | |
|---|---|
| **Evaluation by JECFA**<br>*Reference to the JECFA evaluation (including year and JECFA session of evaluation; full ADI (numerical or "not specified"); specifications monograph).* | *Magnesium Stearate was evaluated by JECFA at the 80th session (Rome, 16–25 June 2015) and an ADI "not specified" was adopted. The summary report has been published (http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/en/) and the Chemical Technical Assessment is also available (http://www.fao.org/3/a-az648e.pdf).*<br>*Specifications will be published in FAO JECFA Monographs 17 and are already available online at http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/* |

**JUSTIFICATION:**

| | |
|---|---|
| **Justification for use and technological need**<br>*Supporting information based on the criteria in Section 3.2 of the Preamble of the General Standard for Food Additives (i.e. has an advantage, does not present an appreciable health risk, serves a technological function).* | *Lubricant / Release agent*<br>*Magnesium stearate is mainly used in the food industry for the production of food supplements and confectionery compressed tablets. In tablet technology, when added to the powder before compression, it acts as a lubricant and assists in the ejection of the tablet from the punch and die. It prevents parts of the tablet sticking to the punches. This function is essential with today's high speed tablet presses as debris build-up on the punches and dies can cause damage. The magnesium stearate also provides a smooth surface to the tablet. The amount of magnesium stearate used in a tablet formula is dependent on the 'stickiness' of the pre-compression granule, but would normally not exceed 3% w/w.*<br>*Anticaking agent*<br>*Magnesium stearate improves the flowability and continuity with its anti-caking effect in certain hydrophobic powdered foods (e.g. spices and herbs) to extend the shelf life of these powders.*<br>*Emulsifier / Binder*<br>*Magnesium stearate can be used as an emulsifier in bakery ware such as rusks and baking powder. It is also used to bind sugar in hard candies like mints.* |

| | |
|---|---|
| | *Magnesium stearate is used by tablet manufacturers worldwide. Over the years, a number of alternative substances have been tried but none is claimed to function as effectively as magnesium stearate. It has been estimated that it is used in around 75% of all food supplement tablets and capsules produced and in over 90% of confectionery tablets. In chewing gums it is estimated to be used up to 30%.*<br><br>*In the European Union, magnesium stearate is included in E470b – Magnesium salts of Fatty Acids, as described by Regulation (EU) No. 231/2012. It can be generally used as additive in foodstuffs with no specific maximum level (quantum satis) as determined by Regulation (EC) No. 1333/2008 on food additives. Excluded are processed foods and foods for which the use of additives is prohibited.*<br><br>*In the Unites States, the affirmation of this ingredient as generally recognized as safe (GRAS) as a direct human food ingredient is based upon the following current good manufacturing practice conditions of use: The ingredient is used as a lubricant and release agent; a nutrient supplement; and a processing aid as defined. And, the ingredient is used in foods at levels not to exceed current good manufacturing practice (§ 184.1440 in CFR 1985).* |
| **Safe use of additive: Dietary intake assessment** (*as appropriate*) | Table 3 additive:<br><br>X Yes<br><br>☐ No (Please provide information on dietary intake assessment below) |
| **Justification that the use does not mislead consumer** | *None of the uses described above is imparting properties to the foods that would be expected by consumers due to other reasons.*<br><br>*Magnesium stearate is part of the INS 470 group of salts of fatty acids which are used and regulated for various purposes without any reported potential to mislead consumers.* |

**(1)** For proposed revisions of adopted provisions, the current adopted provision should be provided, with deletions noted in ~~strikethrough~~ text, and changes or additions noted in **bold** font.

**(2)** Food category number and name, as listed in Annex B of the GSFA.

**(3)** For consistency, the maximum use level should be reported on the same basis as the ADI. A numerical use level should be provided for a food additive assigned a numerical ADI. GMP or a numerical use level may be provided for a food additive assigned a non-numerical ADI (e.g., "not-specified").

**(4)** Comments on specific restrictions on the use of the food additive to be included as Notes (e.g., limitation of use to specific products in a food category).

## INTERNATIONAL ALLIANCE OF DIETARY/FOOD SUPPLEMENT ASSOCIATIONS (IADSA)

| **THE PROPOSAL IS SUBMITTED BY:** | **IADSA (International Alliance of Dietary/Food Supplements Associations)** |
|---|---|
| **IDENTITY OF THE FOOD ADDITIVE:** | |
| **Name of the Additive**<br><br>*As listed in Class Names and the International Numbering System (INS) - CAC/GL 36-1989* | Polyvinyl alcohol (PVA)-polyethylene glycol (PEG) graft co-polymer |
| INS Number | 1209 |
| **Functional Class**<br><br>*As listed in Class Names and the International Numbering System (INS) - CAC/GL 36-1989* | • **Binder**, Carrier, Glazing agent, **Stabilizer**<br><br>**In bold** proposals for additional technological purpose – CX/FA 16/48/14 November 2015 |

| **PROPOSED USE(S) OF THE FOOD ADDITIVE (1):** | | The proposal for ☐ **a new provision**;<br>or ☐revising an existing provision | |
|---|---|---|---|
| **Food Category No. (2)** | **Food Category Name (2)** | **Maximum Use Level (3)** | **Comments (4)** |
| 13.6 | Food supplements | 50,000 mg/kg | Glazing agent (film coating) |
| 13.6 | Food supplements | 100,000 mg/kg | Binder, Stabilizer |

| EVALUATION BY JECFA | |
|---|---|
| **Evaluation by JECFA**<br><br>Reference to the JECFA evaluation (including year and JECFA session of evaluation; full ADI (numerical or "not specified"); specifications monograph). | Year: 2015<br><br>Meeting: 80th (Rome, 16–25 June 2015)<br><br>Specs Code N<br><br>Monograph : 17 (2015) http://www.fao.org/3/a-i5080e.pdf<br><br>Page 47<br><br>ADI:The Committee decided not to establish an ADI "not specified"<br><br>*The summary report is available at the following link: http://www.fao.org/fileadmin/user_upload/agns/pdf/jecfa/Summary_report_of_the_80th_JECFA_meeting.pdf*<br><br>*The Chemical Technical Assessment is also available here http://www.fao.org/3/a-az649e.pdf* |
| **JECFA Summary** The use of PVA-PEG graft co-polymer that complies with the specifications established at the current meeting is not of safety concern when the food additive is used as a glazing agent (aqueous film coating), stabilizer and binder for tablets in the preparation and formulation of food supplements and in accordance with good manufacturing practice | |
| **JUSTIFICATION:** | |
| **Justification for use and technological need**<br><br>*Supporting information based on the criteria in Section 3.2 of the Preamble of the General Standard for Food Additives (i.e. has an advantage, does not present an appreciable health risk, serves a technological function).* | **Glazing agent (film coating)**<br><br>Polyvinyl alcohol-polyethylene glycol-graft-co-polymer (PVA-PEG graft co-polymer) is predominantly intended for use in aqueous instant-release film coatings for food supplement products. When used as a film coating, PVA-PEG graft co-polymer protects against unpleasant tastes or odours, improves appearance, makes the food supplements easier to swallow, and protects the active ingredients in the food supplement. Properties of the co-polymer that are beneficial to its use as a film coating include its low viscosity in aqueous solutions, its high flexibility and elasticity, its rapid dissolution in acidic, neutral, and aqueous media, its ability to reduce the surface tension of water (and thus easier to spray onto food supplements), and its lack of tackiness when formed as a film (which makes it easier to print on). It also has the benefit of being able to form flexible films without the need for an additional plasticizer.<br><br>**Binder, Stabilizer**<br><br>PVA-PEG graft co-polymer can also be used as a binder in rapidly dispersible/soluble granules or tablets, and as a suspension and emulsion stabilizer and protective colloid. |
| **Safe use of additive: Dietary intake assessment (as appropriate)** | The substance is considered by JECFA to be of no safety concern when the food additive is used as a glazing agent (aqueous film coating), stabilizer and binder for tablets in the preparation and formulation of food supplements and in accordance with good manufacturing practice. This conclusion takes into consideration the dietary exposure to ethylene glycol and diethylene glycol and to vinyl acetate from both food supplements and pharmaceutical products.<br><br>The dietary exposure estimate to ethylene glycol and diethylene glycol - from both food supplements and pharmaceutical products - does not exceed 0.016 mg/kg bw per day for children (high consumers). This is 3% of the tolerable daily intake (TDI) of 0.5 mg/kg bw per day derived by the Scientific Committee on Food of the European Union.*<br><br>The dietary exposure estimate to vinyl acetate - from both food supplements and pharmaceutical products - is at least 62 500 times lower than the dose levels at which increases in tumor incidence are observed in oral studies of long-term toxicity and carcinogenicity in rats and mice.<br><br>**EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food). Scientific Opinion on the safety of polyvinyl alcohol-polyethylene glycol-graft-co-polymer as a food additive. EFSA Journal 2013;11(8):3303. 30 pp doi:10.2903/j.efsa.2013.3303)* |
| **Justification that the use does not mislead consumer** | The use of PVA-PEG graft co-polymer fulfills the conditions listed in section 3.2 by preserving the nutritional quality and stability of products and by providing aids in the manufacture of the products. |

| | |
|---|---|
| | Its use is not typically linked with issues related to the nature, freshness, quality of ingredients used or undesirable practices which would mislead the consumer. |

**(1)** For proposed revisions of adopted provisions, the current adopted provision should be provided, with deletions noted in ~~strikethrough~~ text, and changes or additions noted in **bold** font.

**(2)** Food category number and name, as listed in Annex B of the GSFA.

**(3)** For consistency, the maximum use level should be reported on the same basis as the ADI. A numerical use level should be provided for a food additive assigned a numerical ADI. GMP or a numerical use level may be provided for a food additive assigned a non-numerical ADI (e.g., "not- specified").

**(4)** Comments on specific restrictions on the use of the food additive to be included as Notes (e.g., limitation of use to specific products in a food category).

## INTERNATIONAL SPECIAL DIETARY FOODS INDUSTRIES (ISDI)

| | | | |
|---|---|---|---|
| **THE PROPOSAL IS SUBMITTED BY:** | | International Special Dietary Foods Industries (ISDI) | |
| **IDENTITY OF THE FOOD ADDITIVE:** | | | |
| **Name of the Additive**<br>*As listed in Class Names and the International Numbering System (INS) – CAC/GL 36-1989* | | Carrageenan | |
| **INS Number** | | 407 | |
| **Functional Class**<br>*As listed in Class Names and the International Numbering System (INS) – CAC/GL 36-1989* | | Bulking Agent, Carrier, Emulsifier, Gelling Agent, Glazing Agent, Humectant, Stabilizer, Thickener | |
| **PROPOSED USE(S) OF THE FOOD ADDITIVE ([1]):** *The rows below may be copied as many times as needed.* | | The proposal for a<br>☒ new provision; or<br>☐ revising an existing provision. | |
| **Food Category No. ([2])** | **Food Category Name m([2])** | **Maximum Use Level ([3])** | **Comments ([4])** |
| 13.1.1 | Infant formulae | 300 mg/kg | 0.03 g/100 mL in regular milk – and soy-based liquid infant formula (consistent with Codex *Standard for Infant Formulas and Formulas for Special Medical Purposes Intended for Infants* (CODEX STAN 72-1981) |
| 13.1.3 | Formulae for special medical purposes for infants | 1000 mg/kg | 0.1g/100mL in hydrolysed protein- and/or amino acid based liquid infant formula only (consistent with Codex *Standard for Infant Formulas and Formulas for Special Medical Purposes Intended for Infants* (CODEX STAN 72-1981) |
| **EVALUATION BY JECFA:** | | | |
| **Evaluation by JECFA**<br>*Reference to the JECFA evaluation (including year and JECFA session of evaluation; full ADI (numerical or "not specified"); specifications monograph).* | | 79th JECFA Session (2014)<br>"The Committee concluded that the use of carrageenan in infant formula or formula for special medical purposes at concentrations up to 1000 mg/L is not of concern." | |
| **JUSTIFICATION:** | | | |
| **Justification for use and technological need**<br>*Supporting information based on the criteria in Section 3.2 of the Preamble of the General Standard for Food Additives (i.e. has an advantage, does not present an appreciable health risk, serves a technological function).* | | The safety of carrageenan for use in infant formula products has been confirmed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2014.<br>Carrageenan provides a technical effect in infant formula and formulas for special medical purposes which cannot be duplicated by other additives used as stabilizers. | |

| | |
|---|---|
| | • Builds viscosity – Helps to stabilize the sedimentation of dense components such as insoluble calcium and phosphate salts; Slows the upward migration of fat, which is less dense<br>• Deters separation – Without carrageenan for stabilization, formulas would be more likely to produce insoluble sediments or creaming (separation of fat); Assures uniformity of all nutrients throughout shelf life and prevents suboptimal delivery of nutrients<br>• Promotes emulsion – Creating an emulsion during manufacture of formulas made with hydrolyzed proteins would be difficult without carrageenan as oil would immediately separate<br>• Promotes proper mouthfeel – Through proper suspension of insoluble components of formulas, carrageenan creates a smooth, pourable liquid with suitable mouthfeel<br>• Efficacy – Carrageenan does not influence the efficacy of other components in formulas, particularly vitamins and minerals<br>• Lower use needed to achieve function – Carrageenan can be used at lower levels as compared to other stabilizers to achieve the necessary functionality |
| **Safe use of additive: Dietary intake assessment** (*as appropriate*) | Table 3 additive:<br>☐Yes<br>☒No (Please provide information on dietary intake assessment below) |

**Estimation of Carrageenan intake based on consumption data for infant formula**

***HUMAN ESTIMATES OF CARRAGEENAN INTAKE FROM INFANT FORMULA***

JECFA (2008) has published estimates of carrageenan consumption in infants and 12-month old babies for current use levels: 0.03 g/100 ml (300 ppm) for regular milk and soy-based liquid formulas and 0.1 g/100 ml (1,000 ppm) for hydrolyzed protein- and/or amino acid-based liquid formulas. I nfants fed with 100% formula with the carrageenan at 300 ppm and 1,000 ppm show the highest consumption: 47 and 160 mg/kg bw/day, respectively. Estimates for 12-month old babies assume that a caloric intake from infant formula is 13.5% of the total caloric intake; thus, the carrageenan intakes are 6 and 22 mg/kg bw/day for the formula with 300 ppm and 1,000 ppm, respectively (JECFA,2008).

In a human epidemiology study infants were fed formula with 300 ppm carrageenan (Sherry et al., 1993; Sherry et al., 1999). A total carrageenan daily intake of 191 mg/day for the first six months of life was reported (the method of calculation was not published). The mg/kg bw/day intake estimate will depend on the average body weight of a six-month old infant. Using body weight and fluid consumption data from Koletzko et al (2000), it can be estimated that an infant consumes approximately 30.4 mg/kg bw/day carrageenan during the first six months of life from infant formula containing 300 ppm carrageenan. The average carrageenan consumption for the period of 1 to 6 months was estimated as the average of the values for each of the age groups in Table 1.

**TABLE 1: Carrageenan Exposure to Infants from the Infant Formula Application**

| Carrageenan in formula: ppm | mg/kg bw/day | Method of Calculation | Reference |
|---|---|---|---|
| **Regular Milk-Based and Soy-Based Liquid Infant Formula** | | | |
| 300 | 47 | Assumes 100% formula fed infants (100% of caloric intake) | JECFA, 2008 |
| 300 | 6 | Assumes 12-month old infants based on a survey in France showing that consumption of formula represents 13.7% of total caloric intake for this age | JECFA, 2008 |
| 300 | 30.4 | Assumes 100% formula fed infants, one to six months old, using reported body weight data –averaged for 1-6 month olds | Sherry et al., 1993, 1999; Koletzko et al., 2000 |
| 300 | 39.50 | One month old | Calculated based on |

| | | | |
|---|---|---|---|
| | 28.24<br>23.46 | Four month old<br>Six month old | Sherry et al., 1993, 1999; Koletzko et al., 2000 |
| **Hydrolyzed Protein and/or Amino Acid-based Liquid Infant Formula** | | | |
| 1,000 | 160 | Assumes 100% formula fed infants (100% of caloric intake) | JECFA, 2008 |
| 1,000 | 101.2 | Assumes 100% formula fed infants, one to six months old, using reported body weight data – averaged for 1-6 month olds and extrapolated from 300 ppm to 1,000 ppm exposure | Sherry et al., 1993, 1999; Koletzko et al., 2000 |
| 1,000 | 22 | Assumes 12-month old infants, based on a survey in France showing that consumption of formula represents 13.7% of total caloric intake for this age | JECFA, 2008 |

Calculations are on specific age infants: based on Sherry et al., 1993, 1999

In addition, the consumption of carrageenan on a body weight basis (Koletzko et al., 2000) can be calculated as follows.

One Month old: 191 mg/day ÷ 4.835 kg bw = 39.50 mg/kg bw/day Four Month old: 191 mg/day ÷ 6.763 kg bw = 28.24 mg/kg bw/day Six Month old: 191 mg/day ÷ 8.140 kg bw = 23.46 mg/kg bw/day

Thus, the average carrageenan exposures estimated over the time of one to six months is 30.4 mg/kg bw/day as an approximation.

**References:**

JECFA: Joint FAO/WHO Expert Committee on Food Additives. (2008). 68th Meeting of the Joint FAO/WHO Expert Committee on Food Additives; Safety Evaluation of Certain Food Additives and Contaminants, held on June 19-28, 2007 in Geneva, Switzerland. WHO Food Additives Series:59: 65-85.

Sherry, B., Flewelling, A., & Smith, A. L. (1993). Carrageenan: an asset or detriment in infant formula? *Am J Clin Nutr, 58*(5):715.

Sherry, B., Flewelling, A., & Smith, A. L. (1999). Carrageenan: an asset or detriment in infant formula? *Am J Clin Nutr* 58(5): 715, 1993. Erratum: *Am J Clin Nutr, 69*(6):1293.

Koletzko, B., Dokoupil, K., Reitmayr, S., Weinert-Harendza, B., & Keller, E. (2000). Dietary fat intakes in infants and primary school children in Germany. *American J. of Clinical Nutrition, 72* (suppl.):1392S-8S

| | |
|---|---|
| **Justification that the use does not mislead consumer** | Carrageenan is currently used in infant formulas and formulas for special medical purposes around the world. As an ingredient in these products, it is identified on the ingredient list of the product label and does not mislead the consumer. Also, the amount used does not exceed the approved maximum limit and is used as per the technological need indicated above. |

(1) For proposed revisions of adopted provisions, the current adopted provision should be provided, with deletions noted in ~~strikethrough~~ text, and changes or additions noted in **bold** font.

(2) Food category number and name, as listed in Annex B of the GSFA.

(3) For consistency, the maximum use level should be reported on the same basis as the ADI. A numerical use level should be provided for a food additive assigned a numerical ADI. GMP or a numerical use level may be provided for a food additive assigned a non-numerical ADI (e.g., "not-specified").

(4) Comments on specific restrictions on the use of the food additive to be included as Notes (e.g., limitation of use to specific products in a food category).

| | |
|---|---|
| **THE PROPOSAL IS SUBMITTED BY:** | ISDI on behalf of:<br><br>Aaron O'Sullivan<br>Manager, Global Regulatory Affairs<br>Danone Trading Medical BV<br>WTC Schiphol Airport Tower E<br>Schiphol Boulevard 105 |

| | | | |
|---|---|---|---|
| | | 1118 BG Schiphol Airport<br>The Netherlands | |
| **IDENTITY OF THE FOOD ADDITIVE:** Citric and fatty acid esters of glycerol, Citric acid esters of mono- and diglycerides of fatty acids, citroglycerides, mono- and diglycerides of fatty acids esterified with citric acid, CITREM, CAEM; trade name is GRINDSTED® CITREM. INS No. 472c; CAS# 97593-31-2.; E 472c | | | |
| **Name of the Additive**<br>*As listed in Class Names and the International Numbering System (INS) - CAC/GL 36-1989* | | Citric and fatty acid esters of glycerol | |
| **INS Number** | | 472c | |
| **Functional Class**<br>*As listed in Class Names and the International Numbering System (INS) - CAC/GL 36-1989* | | Emulsifier, Antioxidant, Stabilizer | |
| **PROPOSED USE(S) OF THE FOOD ADDITIVE ([1]):** *The rows below may be copied as many times as needed.* | | The proposal for ☑a new provision;<br>or ☐revising an existing provision | |
| **Food Category No. ([2])** | **Food Category Name ([2])** | **Maximum Use Level ([3])** | **Comments ([4])** |
| 13.1 | Infant formulae, follow-on formulae, and formulae for special medical purposes for infants | 0.9 g in all types of liquid infant formula<br>0.75 g in all types of powder infant formula | Maximum level in 100 ml of the product ready for consumption |
| **EVALUATION BY JECFA:** | | | |
| **Evaluation by JECFA**<br>*Reference to the JECFA evaluation (including year and JECFA session of evaluation; full ADI (numerical or "not specified"); specifications monograph).* | | Citric and fatty acid esters of glycerol INS 472 (CITREM) was most recently assessed in 2014. The proposed use is as an emulsifier in food category 13.1: infant formulae, follow-on formulae and formulae for special medical purposes for infants. The output of the assessment was included in 79th report of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), Geneva, 17–26 June 2014.<br>In 2015 the Standard for Infant Formula and Formulas for Special Medical Purposes intended for Infants, CODEX STAN 72 – 1981, was amended to include new provisions for INS 472c. The provisions for CITREM/INS 472c are now listed in Section 4 Table 1 of this standard.<br>Prepared at the 79th JECFA (2014) and published in FAO JECFA. Monographs 16 (2014), superseding specifications prepared at the 35th. JECFA (1989), published in FNP 49 (1990) and in FNP 52 (1992). Metals and arsenic specifications revised at the 61st JECFA (2003). An ADI 'not limited' was established at the 17th JECFA (1973). The specification for lead is under consideration for CCFA 48, 2016. Data has been provided by industry to support this consideration. | |
| **JUSTIFICATION:** | | | |
| **Justification for use and technological need**<br>*Supporting information based on the criteria in Section 3.2 of the Preamble of the General Standard for Food Additives (i.e. has an advantage, does not present an appreciable health risk, serves a technological function).* | | Infant formula, follow on formula and Formulas for Special Medical Purposes intended for Infants manufactured with amino acids and hydrolyzed proteins. Formulations manufactured with amino acids and hydrolyzed proteins have different hydrophobic/hydrophilic characteristics and lower emulsifying capacity than products based on whole protein. CITREM/INS 472c improves the stability and organoleptic properties of products containing (partially) hydrolysed proteins, peptides or amino acids. Emulsifiers are therefore a technological requirement for these formulas to ensure both palatability and prevention of separation of the formula after reconstitution.<br>The JECFA Committee concluded that there are no toxicological concerns about the use of CITREM/INS 472c in infant formula and formula for special medical purposes at concentrations up to 9 g/L. At the higher use levels, there is a possibility of diarrhoea from free citric acid released from formula containing CITREM/INS 472c. Given the | |

| | |
|---|---|
| | paucity of clinical data and the fact that exposure assumptions for citric acid have been maximized, it is difficult to estimate the risk of diarrhoea, but it is considered to be low. Therefore the use of CITREM/INS 472c does not present an appreciable health risk to consumers. |
| **Safe use of additive: Dietary intake assessment** (*as appropriate*) | Table 3 additive:<br>☑ Yes<br>☐ No (Please provide information on dietary intake assessment below) |
| **Justification that the use does not mislead consumer** | In accordance with the provisions of CODEX STAN 72 – 1981, CITREM/INS 472c may be used to produce stable formulations of Infant formula, follow on formula and Formulas for Special Medical Purposes intended for Infants manufactured with amino acids and hydrolyzed proteins. This use servers a technological function as an Emulsifier and ensures the suitability and safety of these formulas for their intended use.<br>Where used, in accordance with CODEX STAN 1-1985 CITREM/INS 472c must be appropriately declared on the label of these products in the list of ingredients by indicating either: (i) the Functional Class together with the specific name or (ii) the Functional Class together with the recognized numerical identification such as the Codex International Numbering System (CAC/GL 36-1989). The placement of CITREM/INS 472c in the ingredients list in descending order must be in accordance with the proportion added to the formula. |

**(1)** For proposed revisions of adopted provisions, the current adopted provision should be provided, with deletions noted in strikethrough text, and changes or additions noted in **bold** font.

**(2)** Food category number and name, as listed in Annex B of the GSFA.

**(3)** For consistency, the maximum use level should be reported on the same basis as the ADI. A numerical use level should be provided for a food additive assigned a numerical ADI. GMP or a numerical use level may be provided for a food additive assigned a non-numerical ADI (e.g., "not-specified").

**(4)** Comments on specific restrictions on the use of the food additive to be included as Notes (e.g., limitation of use to specific products in a food category).

| | | | |
|---|---|---|---|
| **THE PROPOSAL IS SUBMITTED BY:** | | International Special Dietary Foods Industries (ISDI) | |
| **IDENTITY OF THE FOOD ADDITIVE:** | | | |
| **Name of the Additive**<br>*As listed in Class Names and the International Numbering System (INS) – CAC/GL 36-1989* | | Starch sodium octenyl succinate | |
| **INS Number** | | 1450 | |
| **Functional Class**<br>*As listed in Class Names and the International Numbering System (INS) – CAC/GL 36-1989* | | Emulsifier, Stabilizer, Thickener | |
| **PROPOSED USE(S) OF THE FOOD ADDITIVE (1):**<br>*The rows below may be copied as many times as needed.* | | The proposal for a<br>☒ new provision; or<br>☒ revising an existing provision. | |
| **Food Category No. (2)** | **Food Category Name (2)** | **Maximum Use Level (3)** | **Comments (4)** |
| 13.1.3 | Formulae for special medical purposes for infants | 20,000 mg/kg | 2 g/100mL (of product ready for consumption} in hydrolysed protein- and/or amino acid based infant formula only (consistent with Codex Standard for Infant Formulas and Formulas for Special Medical Purposes Intended for Infants (CODEX STAN 72-1981) |
| **EVALUATION BY JECFA:** | | | |
| **Evaluation by JECFA** | | 79th JECFA Session (2014)<br>"The Committee concluded that the use of Starch sodium octenyl succinate in infant formula or formula for special | |

| | |
|---|---|
| *Reference to the JECFA evaluation (including year and JECFA session of evaluation; full ADI (numerical or "not specified"); specifications monograph).* | medical purposes at concentrations up to 20g/L is not of concern." |
| **JUSTIFICATION:** | |
| **Justification for use and technological need**<br>*Supporting information based on the criteria in Section 3.2 of the Preamble of the General Standard for Food Additives (i.e. has an advantage, does not present an appreciable health risk, serves a technological function).* | The safety of starch sodium Octenyl Succinic Acid (OSA-modified starch) for use in infant formula products has been confirmed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2014.<br>OSA-modified starch provides a technical effect in formulas for special medical purposes as an emulsifier as detailed below.<br>• Acts as an emulsifier during the processing of liquid infant formula. Stability of emulsions may need to be maintained for several days before drying. By using OSA-modified starch, the uniformity of the formula composition during drying is ensured.<br>• Acts as an emulsifier after reconstitution of the formula. OSA-modified starch improves the overall dispersability of powder formula<br>• Prevents free fat formation. The use of OSA-modified starch reduces free fat formation through its emulsifying properties. When formulae are not properly emulsified, free fat rises to the top of the formula over time. When a poor emulsion is dried, the free fat passes through the spray nozzle as larger drops of liquid separated from the remainder of the product (rather than being tightly associated with other macronutrients as in a good emulsion). The free fat then adheres to the surface of the dried particles as the particles descend to the bottom of the drier. Once on the surface of the particle, the fat is exposed to oxygen. Oxidation of free fat is undesirable from a nutritional standpoint, as well as from a sensory acceptability standpoint. Additionally, free fat on the particle surface tends to stick to the manufacturing equipment, causing build-up on the surface of the equipment. This results in the requirement for frequent cleaning of equipment and reduced yields. |
| **Safe use of additive: Dietary intake assessment** (*as appropriate*) | Table 3 additive:<br>☐Yes<br>☒ No (Please provide information on dietary intake assessment below) |

**Dietary Intake Determinations for Starch sodium octenyl succinate *International estimates of intake***

Consumption of OSA-modified starch from its use in infant formula was estimated using the World Health Organization (WHO) recommended intakes of milk or infant formula (WHO, 2009) and weight-for-age standards (WHO, 2006) (see Table 1).

**Table 1: Recommended average daily intake of milk or infant formula in infants**

| Age of Infant | Recommended amount of formula per day (mL/kg bw)[a] | Mean body weight (kg)[b] | Total milk or formula per day (mL) |
|---|---|---|---|
| Newborn | 60 | 3.3 | 198 |
| 6 months | 150 | 7.6 | 1,140 |

[a] WHO (2009)

[b] Average of mean body weights for boys and girls (WHO, 2006).

Based on a maximum use level of 20 g/L, the mean intake of OSA-modified starch from its intended use in infant formula and formulae for special medical purposes for infants is estimated to range from 3.96 to 22.8 g per day in infants from birth to six months, as shown in Table 2.

**Table 2: Predicted intakes of OSA-modified starch from its use in infant formula based on WHO recommendations (WHO, 2006)**

| Age of Infant | Average intake (g per day) | Average intake (g/kg bw per day) |
|---|---|---|
| Newborn | 3.96 | 1.2 |
| 6 months | 22.8 | 3 |

WHO = World Health Organization

**Summary and Conclusion**

OSA-modified starch is proposed for use in formulae for special medical purposes intended for infants at levels up to 20 g/L formula. The maximum proposed use-level results in estimated intakes of 1.2 to 3 g/kg bw per day in infants ages zero to six months.

**References**

JECFA (1982). Starch sodium octenyl succinate. In: Toxicological evaluation of certain food additives. 26th Report of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), Apr. 19-28, 1982, Rome, Italy. Geneva, Switz., World Health Organization (WHO). (WHO Food Additives Series No. 17; http://www.inchem.org/documents/jecfa/jecmono/v17je21.htm)

WHO (2006). Weight-for-age standards [construction] (chapter 4). In: Child growth standards: length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: Methods and development. Geneva, Switz., World Health Organization (WHO), WHO Multicentre Growth Reference Study Group, pp. 79–138. (http://www.who.int/childgrowth/standards/technical_report/en/index.html).

WHO (2009). Infant and young child feeding: model chapter for textbooks for medical students and allied health professionals. Geneva, Switz., World Health Organization (WHO). (http://www.waba.org.my/pdf/Infant-n-Young-Feeding.pdf).

| | |
|---|---|
| **Justification that the use does not mislead consumer** | Starch sodium octenyl succinate is currently used in formulas for special medical purposes around the world. As an ingredient in these products, it is identified on the ingredient list of the product label and does not mislead the consumer. Also, the amount used does not exceed the approved maximum limit and is used as per the technological need indicated above. |

**(1)** For proposed revisions of adopted provisions, the current adopted provision should be provided, with deletions noted in strikethrough text, and changes or additions noted in **bold** font.

**(2)** Food category number and name, as listed in Annex B of the GSFA.

**(3)** For consistency, the maximum use level should be reported on the same basis as the ADI. A numerical use level should be provided for a food additive assigned a numerical ADI. GMP or a numerical use level may be provided for a food additive assigned a non-numerical ADI (e.g., “not-specified”).

**(4)** Comments on specific restrictions on the use of the food additive to be included as Notes (e.g., limitation of use to specific products in a food category).