Safety Assessment of N-acetyl-D-neuraminic acid dihydrate (NANA)

Applicant: Glycom A/S

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Novel Food Classification: 1.2.

Introduction

An application for the authorisation of *N*-acetyl-D-neuraminic acid dihydrate (NANA), also known as sialic acid dihydrate, was submitted to the Food Safety Authority of Ireland (FSAI) by Glycom A/S of Denmark in accordance with *Article 4* of the novel food Regulation (EC) No. 258/97. The application was accepted by the FSAI on September 22nd, 2015.

Sialic acid is one of a family of over 60 nine-carbon acidic monosaccharides consisting of either *N* or *O*-substituted derivatives of D-neuraminic acid. While the presence of a mixture of NANA and *N*-glycolyl-neuraminic acid (NGNA) is common in mammalian milk, the occurrence of the pure *N*-acetylated form is a characteristic of human milk. In human breast milk, NANA is predominantly found in the bound form, linked *via* a glycosidic bond to human milk oligosaccharides, proteins (glycoproteins) and lipids (glycolipids). However, it is also present in the free form (10 - 60 mg/L), and it is this form that is chemically and structurally identical to the novel ingredient in this application. NANA is thought to be involved in certain cell signalling events and incorporated into molecules involved in neural development, synaptic transmission, cognition, and memory function, as well as in immune function. This application focuses on the use of free NANA as a novel ingredient and does not address, in any detail, the biological role or fate of bound NANA, or any interaction between the two forms within the body.

The applicant has used x-ray crystallography, ^{1}H- and ^{13}C-NMR as well as mass spectrometry to demonstrate that the novel ingredient is chemically and structurally identical to naturally occurring free NANA. The novel ingredient is produced by coupling *N*-acetylmannosamine and sodium pyruvate to produce anhydrous NANA which undergoes processing, purification, and re-crystallisation to produce the final ingredient. It is intended for use as an ingredient in foods for infants and young children (including infant formula, follow on formula and foods for special medial purposes), other foods for particular nutritional uses, food supplements and general foods including dairy products, cereal bars, beverages and table-top sweeteners. The intended use levels in the various food categories are calculated on the basis of the endogenous levels of free NANA in human breast milk.

NANA has not been added to food in the EU. Though it is chemically and structurally identical to the natural free counterpart, the applicant classes it as novel in accordance

with *Article 1.2(c)* of the novel food Regulation (EC) No 259/97; "Foods and food ingredients with a new or intentionally modified primary molecular structure". The application dossier was prepared pursuant to Commission Recommendation 97/618/EC (Class 1) "pure chemicals or simple mixtures from non-genetically modified sources", (sub-class (2) "the source of the novel food has no history of food use in the Community". However, it could also be considered to fall within *Article 1.2(f)*: "Foods and food ingredients to which has been applied a production process not currently used, where that process gives rise to significant changes in the composition or structure of the foods or food ingredients which affect their nutritional value, metabolism or level of undesirable substances". In that case the application dossier would fall in to Class 6 of Commission Recommendation 97/618/EC: "Foods produced using a novel process", though this would not significantly alter the safety data required.

I. Specification of the novel ingredient

The novel ingredient is a white to off-white crystalline powder with a minimum purity specification of 97%. Batch analyses indicate consistent compliance with specified parameters, including undesirable substances and microorganisms. The applicant presents data from x-ray crystallography, ^{1}H- and ^{13}C-NMR as well as mass spectrometry to demonstrate that the novel ingredient is chemically and structurally identical to free NANA which is naturally present in human breast milk. Interim results from a five year stability study indicate that crystalline NANA stored in specific packaging at 25°C (60% relative humidity), 5°C or -20°C is stable for 36 months. The novel ingredient is also considered to be stable under accelerated conditions (40°C at 70% relative humidity) for up to 24 months, though the applicant considers the ongoing real-time stability study to be a more reliable stability indicator. The stability of NANA was also established under the intended conditions of use in various food matrices including infant formula, yoghurts, beverages and cereal bars.

II. Effect of the production process applied to the novel food

NANA is manufactured in accordance with cGMP and HACCP principles. The applicant provides a detailed description of the manufacturing process which essentially comprises two stages. The first stage involves enzymatic coupling of *N*-acetylmannosamine and sodium pyruvate to produce the starting material, anhydrous NANA. The second stage of the process involves anhydrous NANA undergoing a series of processing steps, including purification, to generate crystalline NANA dihydrate. Quality control procedures are in place for both stages of production and for the final product.

IX. Anticipated intake/extent of use of the novel food

NANA is intended for inclusion in foods for special medical purposes which includes infant and follow-on formula, foods for weight control and gluten free foods. It is also proposed for inclusion in dairy products and analogues, cereal bars, table-top

sweeteners, beverages and food supplements. As summarised in Table 1 below, the proposed maximum use levels of the novel ingredient for infant formula and foods for young children are 50 mg/L for reconstituted infant formula or follow on formula, 50 mg/kg for solid foods and 50 mg/L for other foods for infants and young children.

Table 1: Proposed levels of use in various food categories

Food Category	**Proposed Maximum Use level**
Foods intended for particular nutritional uses	
Foods for infants & young children	
Infant formulae	50 mg/L of reconstituted formula
Follow-on formulae	50 mg/L of reconstituted formula
Processed cereal-based foods & baby foods for infants & young children	50 mg/kg for solid foods
Other foods for young children	50 mg /kg for solid foods 50 mg /L for beverages as consumed
Dietary foods for infants & young children for special medical purposes	Case by case basis
Dietary foods for weight control diets intended to replace total daily food intake or an individual meal	Drinks: 200 mg/L Bars: 1,700 mg/kg
Foods suitable for people intolerant to gluten	500 to 2,500 mg/kg
Dairy products & analogues	
Unflavoured pasteurised & sterilised (incl. UHT) milk	50 mg/L
Unflavoured fermented milk products	Beverages: 50 mg/L Solids: 400 to 800 mg/kg
Unflavoured fermented milk products, heat-treated after fermentation	
Flavoured fermented milk products including heat-treated products	
Dairy analogues, including beverage whiteners	Beverages: 50 mg/L Solids: 250 to 500 g/kg Beverage Whiteners: 16.7 g/kg
Bakery Wares	
Fine Bakery Wares. Cereal Bars Only	500 mg/kg
Sugars, syrups, honey & table-top sweeteners	
Table Top Sweeteners	8,300 mg/kg (8.3 g/kg)
Beverages	
Fruit & vegetable juices	50 mg/L
Fruit & vegetable nectars & similar products	50 mg/L
Flavoured drinks	50 mg/L
Speciality coffee, tea, herbal & fruit infusions, chicory; tea, herbal & fruit infusions & chicory extracts; tea, plant, fruit & cereal preparations for infusions	200 to 400 mg/L
Food supplements	
Food supplements supplied in a solid form including capsules & tablets & similar forms, excluding chewable forms	300 mg/day
Food supplements supplied in a liquid form	
Food supplements supplied in a syrup-type or chewable form	

For the other food categories, a variety of maximum use levels are proposed, ranging from 50 - 200 mg/L for liquid foods and 250 – 8,300 mg/kg for solid foods and table top sweeteners. The supplemental form of NANA (maximum 300 mg/day) is intended as an alternative to regular food sources and so in the applicant's opinion, should not significantly affect daily intakes.

For infants up to six months, the applicant relies on EFSA data on the daily consumption of infant formula (1,060 mL) to estimate a daily intake of 53.1 mg of NANA (8.7 mg/Kg bw/day) with the inclusion of 50 mg of NANA per litre of formula. The level of free NANA in current infant formula (1.3 – 3.3 mg/L) is considered insignificant and therefore not a factor in the calculations. Estimated intakes for infants and young children from infant and follow-on formula, infant-specific foods and foods for young children were based on data from the UK Diet and Nutrition Survey on Infants and Young Children (DNSIYC). At the proposed use level of 50 mg/L for liquids or 50 g/Kg for solids, the applicant estimates the 95th percentile all-user intakes were highest for infants aged between four and six months at 60 mg/person/day (7.0 mg/Kg bw/day) and the lowest for young children aged 13-17 months at 40 mg/person/day (3.5 mg/Kg bw/day).

The levels of NANA proposed for use in foods for other population groups are based on the levels of free NANA in breast milk and utilise data from the UK National Diet and Nutritional Survey (NDNS) and the European Food Safety Authority (EFSA) Food Additive Intake Model (FAIM). Estimates are presented for total food intake (including infant formula but excluding supplements). Women of childbearing age had the highest 95th percentile all-user intakes at 280 mg/day, while children (1 – 10 yr) had the lowest 95th percentile all-user intakes at 140 mg/day. On a body weight basis, toddlers (1 – 3 yr) had the highest 95th percentile all-user consumption at up to 10.7 mg/Kg bw/day depending on age. Male adults and elderly adults had the lowest 95th percentile intakes at 2.2 mg/Kg bw/day.

Food supplements containing NANA (maximum 300 mg/day) are intended as an alternative to NANA containing foods and the applicant estimates a worst case intake scenario would be in adult females aged 19-40 years (5.4 mg/Kg bw/day). High level intakes estimated using the FAIM tool ranged from 2.0 - 9.4 mg/Kg bw/day for adolescents to 13.7 - 33.5 mg/Kg bw/day for toddlers (1 – 3 yr). The applicant however, considered the FAIM estimate to be conservative and prefers estimates derived using the UK survey data from the DNSIYC and NDNS. It is proposed that the conditions laid down for labelling and presentation under food supplements legislation should be sufficient to prevent inadvertent excessive dosing by adults, or any unsupervised use by children.

X. Information from previous human exposure to the novel food or its source

NANA is found in mammalian milk, with the highest concentrations found in human breast milk. Levels of free NANA in human breast milk range between 10 and 60 mg/L depending on lactation period. Low levels of free NANA (1.3 - 3.3 mg/L) are

also present in infant formula deriving from the inclusion of D-lactose. There is also a low level dietary exposure to free NANA through foods like fish and fish eggs, red meat and dairy products.

XI. Nutritional information on the novel food

NANA is a structural component of cell surface oligosaccharide chains of glycolipids that are bound to the cell membrane, with the highest concentrations found in the brain. It is considered a non-caloric monosaccharide since it does not enter the glycolysis pathway and hence has a negligible contribution to energy or nutrient intake. Based on the data provided, there would appear to be little nutritional impact associated with the consumption of free NANA.

XII. Microbiological information on the novel food

The novel ingredient is produced using a combination of chemical and enzymatic processes and so the risk of microbial contamination is low. However, microbiological specifications are set for the product in relation to *Salmonella*, aerobic mesophilic total counts, *Enterobacteriaceae*, *Cronobacter sakazakii*, *Listeria monocytogenes*, *Bacillus cereus*, yeasts, moulds and endotoxins.

XIII. Toxicological information on the novel food

Metabolic fate

A review of the literature on pharmacokinetic and toxicological studies of orally administered NANA is provided. The studies are all carried out in animals and suggest that NANA is almost completely absorbed upon ingestion, with the largest proportion excreted unchanged in the urine. Small amounts of NANA are distributed to the major organs, particularly the liver, brain, and heart, where it may be enzymatically metabolised by *N*-acetylneuraminate lyase to acetylmannosamine and pyruvic acid. Acetic acid also may be formed from the action of *N*-acetylneuraminate lyase together with other enzymes. It is expected that any free NANA not metabolised would be incorporated into the oligosaccharide chains that make up glycolipids, glycoproteins, and human milk oligosaccharides through the endogenous pathway for NANA utilisation. In addition, metabolites like acetylmannosamine would enter a salvage pathway and be converted to NANA.

Acute Studies

No acute toxicity studies on NANA were performed, or identified from the literature.

Repeated Exposure Studies

In a sub-chronic oral toxicity study in rats, NANA was found to be without maternal toxicity or compound-related adverse effects on female reproduction as well as the general growth and development of offspring at maternal dietary concentrations of up to 2% (1,895 mg/kg bw/day) up to 7 weeks. Compound related adverse effects were not observed in a 13 week dietary toxicity study in F_1 pups. An overall NOAEL for

NANA in rats was determined by the applicant to be 1,246 mg/kg bw/day, the highest dose tested.

Mutagenicity and Genotoxicity Studies

NANA was non-mutagenic in the bacterial Ames test at concentrations up to 5,000 µg/plate, and in a micronucleus assay with human peripheral blood cells (up to 3,450 µg/mL +/- S9), compared to negative controls.

Oral Studies

Three animal studies reported in the literature investigated short term exposure to NANA, with no adverse effects reported. One study involved administration of 1% NANA (714 mg/kg bw/day) in the diet of 8-week-old male Sprague-Dawley rats for 2 weeks. Another involved 3-day-old male domestic piglets (Landrace/Large White cross) receiving either a control pig milk replacer or a NANA supplemented milk replacer, with NANA concentrations representing approximate intakes of 40, 85, 180, and 240 mg/kg bw/day. The third study investigated the effects of maternal dietary NANA supplementation in n-3 fatty acid-deficient female Wistar rats on the learning abilities of F1 offspring after weaning.

Allergenicity

Allergenicity is not considered an issue due to the chemical and enzymatic nature of the production process and as protein is generally below the limit of detection in the final product.

Conclusions

NANA has not previously been added to any foodstuffs in the EU and therefore requires pre-authorisation as a novel food ingredient under the novel food Regulation. The novel ingredient is chemically and structurally identical to the naturally occurring free NANA found in mammalian milk (highest in human breast milk) and at relatively low levels in a number of foods. The fate of ingested free NANA in animals is relatively well characterised, though a definitive biological role has yet to be elucidated. Nevertheless, the applicant works on the assumption that the natural presence of free NANA in human breast milk means it is a beneficial if not necessary ingredient for the growth and development of the most vulnerable population. The proposed use levels in various foodstuffs are described in detail and are developed on the basis of free NANA levels in breast milk, while incorporating intake data from a number of sources. Though not specifically targeted at any sub-population by the applicant, supplements containing NANA are intended to replace other foods rather than be consumed in conjunction with other NANA-containing foods. The regulatory regimes associated with the use of food supplements in the EU are considered sufficient safeguards by the applicant to prevent over consumption of NANA by adults while avoiding any unsupervised exposure to children. Hypothetical over-consumption scenarios by adults are considered by the applicant without raising any significant safety concerns.

The toxicological profile of NANA has been adequately investigated by the applicant and no safety concerns of a toxicological nature have been identified.

Recommendation

The Food Safety Authority of Ireland has not identified any safety concerns associated with the consumption of N-acetyl-D-neuraminic acid dihydrate (NANA) at the proposed use levels in foods or food supplements containing the novel ingredient. Therefore the FSAI is of the opinion that it meets the criteria for novel food set out in *Article 3.1.* of the novel food Regulation (EC) No 258/97.