

Safety Assessment of 2'-O-Fucosyllactose (2'-FL)

Name of Applicant: Glycom A/S

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Novel Food Classification: 1.2.

Introduction

An application for the authorisation of 2'-O-Fucosyllactose (2'-FL) was submitted to the Food Safety Authority of Ireland (FSAI) by Glycom A/S from Denmark in accordance with *Article 4* of the novel food Regulation (EC) No. 258/97. The application was accepted by the FSAI on June 20th, 2014.

The novel ingredient is a trisaccharide consisting of L-fucose, D-galactose and D-glucose and is identical to a trisaccharide naturally present in most human breast milk. A recent EFSA Opinion cites fucosyllactoses (of which 2'-FL is a member) as one of about 20 oligosaccharides that make up approximately 90% of the oligosaccharide content in human milk. It is notable that a percentage of women do not have a particular enzyme which is critical for the biosynthesis of the naturally occurring 2'-FL in the mammary gland. There is currently no evidence of a direct nutritional role for this trisaccharide in humans. The major proportion of ingested 2'-FL reaches the large intestine where it is primarily metabolised by intestinal microflora, with a small proportion absorbed and then excreted in urine.

The applicant intends to market the novel ingredient in a number of general foodstuffs, food supplements and some foods for particular nutritional uses (PARNUTS) including infant formula and follow-on formula. This ingredient has not been used in food production previously in the EU and is classed by the applicant 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 and in order to assess wholesomeness, 2'-FL was considered in Class 1 "pure chemicals or simple mixtures from non-genetically modified sources", and sub-class (2) "the source of the novel food has no history of food use in the Community".

I. Specification of the novel food

The applicant provides comprehensive information about the identity of the novel ingredient, concluding that it is identical to the naturally occurring 2'-FL found in human breast milk. The novel ingredient is produced as a white to off-white powder with a minimum specified purity of 95.0% (HPLC analysis), though batch analysis puts the actual purity at $\geq 97\%$ on average. Protein content is specified at a maximum

level of 0.1%, but batch analysis indicates that protein content is generally below the limit of quantification. Solvent residues as well as heavy metal and microbiological contaminants are controlled by specifications. The stability of crystalline 2'-FL for 36 months (under ambient conditions and controlled humidity) and six months (under accelerated conditions) has been demonstrated. The applicant also provides data from ongoing studies on the stability of the novel ingredient in certain food matrices including infant formula powder, yoghurt, juice and flavoured milk.

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

2'-FL is produced through a series of well-defined physical and chemical reactions subject to good manufacturing practices and in line with HACCP principles. Benzyl-2'-FL is designated by the applicant as the primary raw material and is derived from L-fucose and D-lactose as starting raw material in Stage 1 of the production process. The novel ingredient (2'-FL) is then produced in Stage 2 of the process, with quality control checks in place throughout the process.

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

The novel ingredient is intended for use in PARNUTS, including FSMPs as well as infant and follow-on formula, among other children's foods. It will also be added to a range of general foodstuffs including dairy products and analogues, cereal bars, syrups, sweeteners, beverages and dietary supplements. The applicant included a comprehensive analysis of intake estimates using data from four sources: (a) EFSA estimates of infant formula intake; (b) UK Diet and Nutrition Survey of Infants and Young Children (DNSIYC 2011) for estimates of infant formulae and foods specifically designed for young children only, (c) UK National Diet and Nutrition Survey programme to estimate total dietary intake; (d) EFSA 'Food Additives Intake Model' (FAIM) for estimates of total food intakes in 17 EU countries using data from 26 dietary surveys. As the FAIM tool offers very conservative estimates of exposure, the applicant excluded this data from the safety assessment, relying primarily on UK data sources.

The maximum levels of 2'-FL proposed for all food categories are based on the intake by infants from mature breast milk. This is estimated by the applicant at an average of 170-660mg/kg body weight/day, up to a maximum of 1,150mg/kg body weight/day for a 6.5Kg infant drinking approximately 1L of breast milk per day. Intake estimates are based on the "worst case" scenario where the novel food replaces all existing similar foods and safety was assessed by comparison with concentrations of 2'-FL naturally found in human milk, along with an estimated NOAEL of 5,000mg/kg body weight/day. EFSA has estimated the average daily intake of liquid infant formula at 1,060 mL/day for infants aged 0-6 months, based on a 3 month old infant weighing 6.1Kg consuming 174 mL/kg body weight (bw)/day at the 95th percentile of intake.

The applicant intends to use 2'-FL in supplement form at up to 3g/day as an alternative to food use and does not expect this to add to the overall daily intake from food.

Summary table of proposed intakes of 2'-FL for different population groups

Population Group	Estimated 95 th percentile intake (maximum use level) (mg/kg bw/d)
Infants (0-6 months formula only)	417.6
DNSIYC (all users of infant formulae & foods specifically designed for young children only)	
Infants (4-6 months)	668
Infants (7-12 months)	641
Young children (13-17 months)	355
UK NDNS (proposed food uses for all users)	
Toddlers (1-3 yr)	247
Children (4-10 yr)	135
Teenagers (11-18 yr)	55
Women of child bearing age (19-40 yr)	75
Female adults (19-64 yr)	74
Male adults(19-64 yr)	50
Elderly adults (≥65 yr)	55

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

2'-FL is one of the naturally occurring fucosylated oligosaccharides found in the milk of some mammalian species including humans, cows, goats and sheep. 2'-FL is present at highest concentrations in human milk, though it is not detected in women lacking the enzyme 1,2-fucosyltransferase in their mammary glands. The level in human breast milk varies considerably between individuals (1,100 – 4,260 mg/L) and is generally at its highest in colostrum. 2'-FL is part of a complex mixture of more than a hundred oligosaccharides found in human milk and which the applicant claims may have a prebiotic role. Though the addition of other oligosaccharides such as fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS) to infant formula is permitted in the EU, 2'-FL is not currently added specifically as an ingredient to any foodstuffs in the EU. Therefore, human exposure to 2'-FL has been limited to its natural occurrence in human breast and other milks including that of cows, goats and sheep which have lower concentrations.

XI. Nutritional information on the novel food

Consumption of 2'-FL at the levels proposed and in the food categories intended is not expected to have a significant nutritional impact. The novel ingredient does not undergo significant metabolism or absorption in the upper gastrointestinal tract but is instead metabolised through fermentation by bacteria residing in the large intestine or excreted in the faeces. For this reason, the novel ingredient is not likely to be of any direct nutritional or calorific value to humans, and is intended by the applicant to be a source of selective nutritional support for certain members of the gut microflora.

XII. Microbiological information on the novel food

The microbiological status of the novel ingredient, including endotoxin content is controlled via product specifications and supported by batch test results.

XIII. Toxicological information on the novel food

The applicant noted there were no acute studies on 2'-FL evident in the scientific literature.

Repeated Exposure Studies

An adapted subchronic (90-day) oral toxicity study was carried out in juvenile rats to determine the potential toxicity of the novel ingredient. The main study was preceded by a 14-day dose-range finding study which determined 6,000 mg/kg bw/day by gavage of 2'-FL (purity 99%) to be suitable as the highest test dose. Fructo-oligosaccharides (FOS) were used in the control (reference) group. A possible treatment-related causation could not be discounted for mortalities recorded at the highest dose. The conclusion by the investigators was that the oral administration of up to 5,000 mg/kg body weight/day (NOAEL) of 2'-FL in juvenile rats for 13 weeks was well tolerated and that any adverse effects recorded (lower body weight gain and coloured liquid faeces) were transient.

Mutagenicity

The mutagenic potential of 2'-FL (99% purity) was investigated using the Ames test employing both the plate incorporation and pre-incubation methods. The novel ingredient was deemed to be non-mutagenic at concentrations up to 5,000 µg/plate. In addition, a mammalian cell gene mutation assay using L5178Y tk+/- mouse lymphoma cells did not reveal statistically or biologically significant mutagenic effects of 2'-FL at concentrations up to 5,000 µg/ml.

Human Studies

The applicant did not identify any human studies relating to 2'-FL in the literature.

Allergenicity

Allergenicity is not considered an issue as protein was not detected in the novel ingredient.

Conclusions

2'-O-Fucosyllactose (2'-FL) is a natural constituent of mammalian milk, with human breast milk having the highest levels among animal species, in particular colostrum. The novel ingredient in this application is identical to the corresponding constituent in human breast milk, and is derived from L-fucose and D-lactose through a series of chemical and physical interactions.

The applicant has identified the food types in which they intend to market the food. Based on food intake data from a number of sources, potential average intakes were estimated and no nutritional concerns were identified. The toxicological data provided has not identified any concerns regarding the safety of this ingredient when consumed as intended, even by vulnerable groups such as infants and young children.

Recommendation

The Food Safety Authority of Ireland has not identified any safety concerns associated with the consumption of 2'-*O*-Fucosyllactose (2'-FL) in the proposed food groups and at the intended use levels and therefore considers that it meets the criteria for novel food set out in *Article 3.1.* of the novel food Regulation (EC) No 258/97.