SCIENTIFIC OPINION

ADOPTED: 29 June 2015

PUBLISHED: 20 July 2015

doi:10.2903/j.efsa.2015.4184

Safety of 2'-*O*-fucosyllactose as a novel food ingredient pursuant to Regulation (EC) No 258/97

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)

Abstract

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on 2'-*O*-fucosyllactose as a novel food ingredient (NFI) submitted pursuant to Regulation (EC) No 258/97 of the European Parliament and of the Council, taking into account the comments and objections of a scientific nature raised by Member States. 2'-*O*-fucosyllactose (2'-FL) is a synthetic trisaccharide, which is intended to be used in infant and follow-on formulae, foods for special medical purposes for infants and young children, and other foods for infants and young children, as well as in foods or food supplements for adults. The information provided on the potential mutagenicity of 2'-FL does not raise safety concerns as regards the genotoxicity of this NFI. Based on the observations from a sub-chronic 90-day toxicity study in rats, the Panel considers that the no observed adverse effect level is 2 000 mg/kg body weight per day. The applicant provided a double-blind, randomised, controlled clinical trial on the effects of 2'-FL consumed in combination with another oligosaccharide (lacto-*N*-neotetraose (LNnT)) in infants. The Panel concludes that 2'-FL is safe for infants (up to one year of age) when added to infant and follow-on formulae, in combination with LNnT, at concentrations up to 1.2 g/L of 2'-FL and up to 0.6 g/L of LNnT, at a ratio of 2:1 in the reconstituted formulae; is safe for young children (older than one year of age) when added to follow-on and young-child formulae, at concentrations up to 1.2 g/L of 2'-FL (alone or in combination with LNnT, at concentrations up to 0.6 g/L, at a ratio of 2:1). The Panel also concludes that 2'-FL is safe when added to other foods at the uses and use levels proposed by the applicant.

Keywords: 2'-*O*-fucosyllactose, 2'-FL, synthetic oligosaccharide, novel food ingredient, safety

Requestor: European Commission following an application by Glycom A/S

Question number: EFSA-Q-2015-00052

Correspondence: nda@efsa.europa.eu

Panel members: Carlo Agostoni, Roberto Berni Canani, Susan Fairweather-Tait, Marina Heinonen, Hannu Korhonen, Sébastien La Vieille, Rosangela Marchelli, Ambroise Martin, Androniki Naska, Monika Neuhäuser-Berthold, Grażyna Nowicka, Yolanda Sanz, Alfonso Siani, Anders Sjödin, Martin Stern, Sean (J.J.) Strain, Inge Tetens, Daniel Tomé, Dominique Turck and Hans Verhagen.

Acknowledgements: The Panel wishes to thank the members of the Working Group on Novel Foods: Paul Brantom, Karl-Heinz Engel, Marina Heinonen, Hannu Korhonen, Rosangela Marchelli, Bevan Moseley, Monika Neuhäuser-Berthold, Annette Pöting, Morten Poulsen, Seppo Salminen, Josef Schlatter, Hendrik Van Loveren and Hans Verhagen for the preparatory work on this scientific opinion.

Suggested citation: EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2015. Scientific opinion on the safety of 2'-*O*-fucosyllactose as a novel food ingredient pursuant to Regulation (EC) No 258/97. EFSA Journal 2015;13(7):4184, 32 pp. doi:10.2903/j.efsa.2015.4184

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

Summary

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on 2'-*O*-fucosyllactose as a novel food ingredient submitted pursuant to Regulation (EC) No 258/97 of the European Parliament and of the Council, taking into account the comments and objections of a scientific nature raised by Member States. The assessment follows the methodology set out in Commission Recommendation 97/618/EC of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients (NFIs) and the preparation of initial assessment reports under Regulation (EC) No 258/97. The assessment is based on the data supplied in the original application, the initial assessment by the competent authority of Ireland, the concerns and objections of the other Member States and the responses of the applicant.

2'-*O*-fucosyllactose (2'-FL) is a synthetic trisaccharide consisting of L-fucose, D-galactose and D-glucose, which is produced by using L-fucose and D-lactose as starting raw materials. The NFI is intended by the applicant to be used in infant and follow-on formulae, foods for special medical purposes for infants and young children, and other foods for infants and young children. The NFI is also intended to be used in foods or food supplements for adults. The Panel considers that the information provided on the specifications, the data from batch testing and the production process do not raise safety concerns.

Based on food consumption data of EFSA for infants and the use level of the NFI (2.4 g/L) proposed by the applicant, 2'-FL intakes from infant formula would result in about 2.5 g per day and about 418 mg/kg body weight, respectively, at the 95th percentile for a 3-month-old infant weighing 6 kg. According to the intake estimate provided by the applicant based on individual consumption data from the UK, 2'-FL intakes from infant formula could lead to an intake of about 670 mg/kg body weight per day at the 95th percentile for infants aged from 4 to 6 months.

For other population groups, the applicant estimated in a tiered approach, intakes by using the EFSA Food Additive Intake Model tool based on summary statistics of consumption data of the EFSA Comprehensive Food Consumption Database. For a refined intake estimate, the applicant used individual consumption data recorded by the UK National Diet and Nutrition Survey for the years 2008–2010.

Considering that 2'-FL is a naturally occurring trisaccharide that is present in human milk, the history of human exposure to 2'-FL is limited primarily to that of breast-fed infants. The Panel notes that the proposed maximum intake level of the NFI by infants is within the range of 2'-FL levels found in mature breast milk, as reported in the literature, although at the high end of this range. Taking into account that there are no indications that the absorption of the NFI may differ from that of 2'-FL from breast milk, the Panel considers that the limited amount of NFI that could be absorbed does not raise safety concerns.

The applicant provided three studies on the potential mutagenicity of 2'-FL (a bacterial reverse mutation assay, an *in vitro* mammalian cell gene mutation test and an *in vitro* micronucleus assay). The Panel considers that the information provided does not raise safety concerns as regards the genotoxicity of the NFI.

Based on the observations from a sub-chronic 90-day toxicity study in rats, the Panel considers that the no observed adverse effect level is 2 000 mg/kg body weight per day.

The applicant provided an unpublished study report on a double-blind, randomised, controlled clinical trial on the effects of a combination of two oligosaccharides, 2'-FL and lacto-*N*-neotetraose (LNnT), which are both under safety assessment as NFIs, in infants. This study reported that the weight gain of infants who consumed the formula with added 2'-FL and LNnT was not inferior to the weight gain of infants who were fed the control formula up to four months of age. The results on stool endpoints and the reported changes in the composition of microbiota in infants consuming the combination of the two oligosaccharides do not raise safety concerns for the studied combination. The Panel considers that this study can be used to draw conclusions on the safety of this combination at the concentrations tested (i.e. 1.0–1.2 g/L of 2'-FL and 0.5–0.6 g/L of LNnT for reconstituted formula).

Noting that complementary feeding of infants and young children results in an increasingly diversified diet, the Panel considers that the potential impact of the addition of 2'-FL alone (without LNnT) or in combination with LNnT on the development of the microbiota diminishes with the proportion of complementary feeding and with age. The Panel therefore considers that 2'-FL alone or in combination with LNnT added at the proposed concentration in foods intended for children older than one year of age, does not raise safety concerns.

The applicant also provided an unpublished study report on a placebo-controlled, double-blind, parallel, dose-response trial, in healthy adults who were randomised to consume either 2'-FL or LNnT, alone or in different combinations, or placebo for two weeks. This study showed that participants who consumed daily 20 g of 2'-FL reported a significant increase in nausea, rumbling, bloating, passing gas, diarrhoea, loose stools and urgency compared with the placebo group, while, for subjects consuming 10 g of 2'-FL per day, these effects were not observed. Considering the highest estimated 95th percentile daily intake of 2'-FL of 75 mg/kg body weight and 5 g per person, respectively, in women of child-bearing age plus the maximum intended daily intake from food supplements of 3 g, 2'-FL intakes in this population group could reach up to 8 g per day.

The Panel concludes that the NFI, 2'-FL, is safe for infants (up to one year of age) when added to infant and follow-on formulae, in combination with LNnT, at concentrations up to 1.2 g/L of 2'-FL and up to 0.6 g/L of LNnT, at a ratio of 2:1 in the reconstituted formulae; is safe for young children (older than one year of age) when added to follow-on and young-child formulae, at concentrations up to 1.2 g/L of 2'-FL (alone or in combination with LNnT, at concentrations up to 0.6 g/L, at a ratio of 2:1). The Panel also concludes that 2'-FL is safe when added to other foods at the uses and use levels proposed by the applicant.

Table of contents

1. Introduction

1.1. Background and Terms of Reference as provided by the European Commission

On 23 June 2014, the company Glycom A/S submitted a request in accordance with Article 4 of the Novel Food Regulation (EC) No 258/97[1] to place on the market 2'-*O*-fucosyllactose (2'-FL) as a novel food ingredient.

On 3 October 2014, the competent authority of Ireland forwarded to the Commission its initial assessment report, which came to the conclusion that 2'-FL meets the criteria for acceptance of a novel food defined in Article (3)1 of Regulation (EC) No 258/97.

On 9 October 2014, the Commission forwarded the initial assessment report to the other Member States. Several Member States submitted comments or raised objections.

In consequence, a decision is now required by the Commission under Article 7(1) of Regulation (EC) No 258/97.

The concerns of a scientific nature raised by the Member States can be summarised as follows:

- The product specification allows for a lower level of purity, compared to the product that was used for actual experiments (≥ 95 % as opposed to > 99 % in the 90 day toxicity study in rats). The applicant should make sure that the actual product does not contain more contaminants than the test article used to obtain the necessary experimental safety data, in order to vouch for the validity of the safety data recorded in the dossier.
- Regarding the microbiological parameters in the specification, specific conditions for several types of foods are more extensively described in Regulation 2073/2005, as amended. In particular, this has implications for the sampling plans for final products that would be formulated to contain 2'-FL.
- Clarification was requested on the accreditation of the laboratories which carried out the analyses.
- A detailed chemical characterisation of the product is needed.
- Given that an organometallic catalyst is used in the manufacturing process, it would be useful for heavy metals analyses to be included as part of the quality assurance procedure.
- In the dossier, it is stated that the intake of 2'-FL in the form of food supplements would not be expected to influence the overall daily intake. However, one should consider the possibility for combined intake of 2'-FL from food supplements and other food uses specified in the dossier as a realistic scenario, that could more or less double the daily intake for high-level users.
- Intake of large quantities of indigestible carbohydrates may produce laxative effects. As there are no human studies on 2'-FL, apart from experience with infants who have been breast-fed, nothing can be said about the quantities of 2'-FL which may produce laxative effects.
- Considering that the novel food ingredient may also be consumed by more vulnerable individuals such as those with conditions that result in a 'leaky gut', any further information could be provided on any possible consequences that may be associated with an increased level of absorption of the novel food ingredient.
- The potential for genotoxicity was only assessed in two *in vitro* tests for mutagenicity. A test for potential clastogenic activity is lacking (*in vitro* micronucleus test or *in vitro* chromosome aberration test). The absence of genotoxicity for this 2'-FL-preparation would only be demonstrated sufficiently, upon obtaining a favourable outcome in such study.
- No data on human trials using the applicant's 2'-FL are reported in the dossier. Whereas the application in infant formula and follow-on formula would mirror the endogenous presence of 2'-FL in human milk, the applications for other age groups lack such a basis for comparison of

[1] Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. OJ L 43, 14.2.1997, p. 1–6.

tolerability for 2'-FL ingestion. In particular, this could be relevant for the consumption of 2'-FL in concentrated forms, as could be the case for food supplements.

- Additional information is needed on the reason behind the use of this NFI.

In accordance with Article 29(1)(a) of Regulation (EC) No 178/2002,[2] the European Food Safety Authority is asked to carry out the additional assessment for 2'-*O*-fucosyllactose as a novel food ingredient in the context of Regulation (EC) No 258/97.

EFSA is asked to carry out the additional assessment and to consider the elements of a scientific nature in the comments raised by the other Member States.

2. Data and Methodologies

2.1. Data

The assessment of the safety of this NFI is based on data supplied in the original application, the initial assessment by the competent authority of Ireland, the concerns and objections of the other Member States and the responses of the applicant (see 'Documentation provided to EFSA').

In accordance with Commission Recommendation 97/618/EC,[3] 2'-*O*-fucosyllactose (2'-FL) is allocated to Class 1.2, i.e. 'pure chemicals or simple mixtures from non-genetically modified sources; the source of the NF has no history of food use in the Community'. The data are required to comply with the information required for novel foods of Class 1.2, i.e. structured schemes I, II, IX, X, XI, XII and XIII of Commission Recommendation 97/618/EC. In the current opinion, these structured schemes are listed in sections 3.1 to 3.8. The applicant's intention is to use the NFI in infant formula (IF), follow-on formula (FOF), foods for special medical purposes for infants and young children, and other foods for infants and young children. The applicant also proposed the use of this NFI in foods or food supplements for adults. This assessment concerns only risk that might be associated with consumption, and is not an assessment of the efficacy of 2'-FL with regard to any claimed benefit.

2.2. Methodologies

The assessment follows the methodology set out in Commission Recommendation 97/618/EC of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and NFIs and the preparation of initial assessment reports under Regulation (EC) No 258/97 of the European Parliament and of the Council.

[2] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–24.

[3] 97/618/EC: Commission Recommendation of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients and the preparation of initial assessment reports under Regulation (EC) No 258/97 of the European Parliament and of the Council. OJ L 253, 16.9.1997, p. 1–36.

3. Assessment

3.1. Specification of the Novel Food Ingredient (NFI)

The NFI is 2'-*O*-fucosyllactose (2'-FL), a synthetic oligosaccharide produced using L-fucose and D-lactose as starting raw materials (section 3.2).

2'-FL is a trisaccharide consisting of L-fucose, D-galactose and D-glucose (chemical formula: $C_{18}H_{32}O_{15}$; molecular weight: 488.44 Da; CAS No 41263-94-9) (Figure 1). The structure can be described as consisting of the monosaccharide L-fucose and the disaccharide D-lactose, which are linked by an α-(1→2) bond to form the trisaccharide.

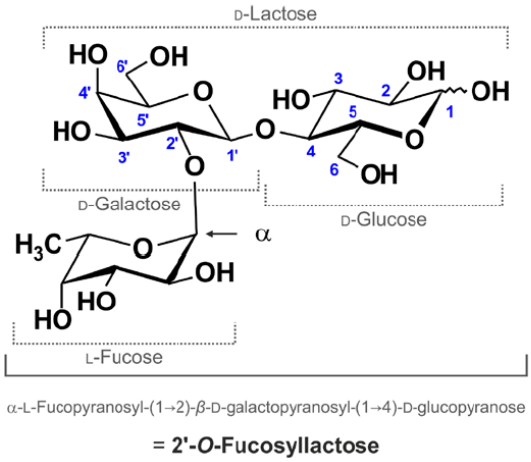

Figure 1: Molecular structure of 2'-FL

The IUPAC name of 2'-FL is α-L-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→4)-D-glucopyranose. Alternative names are 2'-*O*-L-fucosyl- D-lactose; fucosyl-α-1,2-galactosyl-β-1,4-glucose; and Fuc-α-(1→2)-Gal-β-(1→4)-Glc.

2'-FL has been characterised by spectroscopic techniques (e.g. ^{1}H-, ^{13}C-, and 2D-nuclear magnetic resonance (NMR)), mass spectrometry (MS) and high-performance liquid chromatography (HPLC). The chemical and spectroscopic data provided by the applicant are consistent with the structure proposed (Ishizuka et al., 1999). The applicant demonstrated that the NFI is identical to the 2'-FL that is present in human breast milk by comparison with an authentic specimen. In response to a comment from a Member State, the applicant provided additional data on the chemical structure of 2'-FL.

The product specifications, as proposed by the applicant, are presented in Table 1.

Analytical results from five batches of the NFI, which complied with the product specifications, are presented in Table 2.

Table 1: Specifications for 2'-FL as proposed by the applicant

Parameter	Specification	Method
Appearance	Powder	MSZ ISO 6658:2007
Colour	White to off-white	MSZ ISO 6658:2007
Identification	RT of standard ± 3%	Glycom method HPLC-202-2C4-003
2'-FL (water free)	Min. 95.0 %	Glycom method HPLC-202-C4-002
D-lactose[(a)]	Max. 1.0 w/w %	Glycom method HPAEC-202-001
L-Fucose[(a)]	Max. 1.0 w/w %	Glycom method HPAEC-202-001
Difucosyl-D-lactose isomers[(a)]	Max. 1.0 w/w %	Glycom method HPAEC-202-001
2'-Fucosyl-D-lactulose[(a)]	Max. 0.6 w/w %	Glycom method HPLC-202-2C4-003
pH (20°C, 5 % solution)	3.2 to 7.0	EP method
Water	Max. 9.0%	Karl-Fischer (EP 2.5.32)
Ash, sulphated	Max. 0.2%	EP 2.4.14
Acetic acid[(b)]	Max. 0.3%	MSZ EN ISO 10304-1:2009
Residual solvents	Max. 50 mg/kg singly Max. 200 mg/kg in combination	EP GC 2.4.24
Residual proteins[(c)]	Max. 0.01 %[(d)]	Bradford protein assay
Palladium	Max. 0.1 mg/kg	ICP-MS EPA 6020A:2007
Nickel	Max. 3.0 mg/kg	ICP-MS EPA 6020A:2007
Microbiological specifications		
Salmonella	Absent in 25 g	MSZ-EN-ISO 6579:2006
Aerobic mesophilic total count	Max. 500 CFU/g	MSZ-EN-ISO 4833:2003
Enterobacteriaceae	Absent in 10 g	MSZ-ISO 21528-2:2007
Cronobacter (Enterobacter) sakazakii	Absent in 10 g	ISO-TS 22964:2006
Listeria monocytogenes	Absent in 25 g	MSZ-EN-ISO 11290-1:1996, 1998/A1:2005
Bacillus cereus	Max. 50 CFU/g	MSZ-EN-ISO 7932:2005
Yeasts	Max. 10 CFU/g	MSZ-ISO 7954:1999
Moulds	Max. 10 CFU/g	MSZ-ISO 7954:1999
Residual endotoxins[(c)]	Max. 10 EU/mg[(d)]	EP 2.6.14

CFU: colony forming units; EP: European Pharmacopoeia; EPA: Environmental Protection Agency; EU: endotoxin units; GC: gas chromatography; HPLC: high-performance liquid chromatography; HPAEC: high-performance anion exchange chromatography; ICP-MS: inductively coupled plasma mass spectrometry; max.: maximum; min.: minimum; RT: retention time

(a): These specifications refer to the water-free compound

(b): As free acid and/or sodium acetate

(c): Specifications for residual proteins and endotoxins are included to allow control on any impurity stemming from potential raw materials produced from alternative production processes (i.e. fermentation) and to provide reassurance that 2'-FL produced through Glycom's method of manufacture is controlled for residual levels of proteins and endotoxins.

(d): Specification may be amended (i.e. lowered) should additional data become available in the future.

Table 2: Analyses of five batches of the NFI

Parameter	Specification	Batch results				
		L06112K	**PSD4201 31210/166**	**PSD4201 31210/167**	**PSD4201 31210/168**	**PSD4201 31210/169**
Appearance	Powder	Powder	Powder	Powder	Powder	Powder
Colour	White to off-white	White	White	White	White	White
Identification	RT of main component corresponds to RT of standard ± 3%	Complies	Complies	Complies	Complies	Complies
2'-FL (water free) (%)	Min. 95.0 %	96.9	97.0	98.2	97.0	97.8
D-Lactose (%)[(a)]	Max. 1.0 w/w %	0.07	0.09	0.10	0.15	0.18
L-Fucose (%)[(a)]	Max. 1.0 w/w %	< LOR[(f)]	0.03	0.04	0.06	0.08
Difucosyl-D-lactose isomers (%)[(a)]	Max. 1.0 w/w %	< LOR[(f)]	< LOR[(f)]	< LOR[(f)]	< LOR[(f)]	< LOR[(f)]
2'-Fucosyl-D-lactulose (%)[(a)]	Max. 0.6 w/w %	0.06	0.42	0.49	0.35	0.30
pH (20°C, 5% solution)	3.2 to 7.0	6.2	3.9	3.9	3.7	3.6
Water (%)	Max. 9.0	0.3	2.8	3.2	3.2	3.1
Ash, sulphated (%)	Max. 0.2	0.15	< 0.01	0.07	0.05	0.05
Acetic acid (%)[(b)]	Max. 0.3	0.003	0.05	0.05	0.03	0.03
Residual solvents Singly (mg/kg) Combination (mg/kg)	 Max. 50 Max. 200	 n/a[(d)] n/a[(d)]	 < 10 < 70	 < 10 < 70	 < 10 < 70	 < 10 < 70
Residual proteins (%)[(e)]	Max. 0.01[(e)]	< LOQ[(g)]	< LOQ[(g)]	< LOQ[(g)]	< LOQ[(g)]	< LOQ[(g)]
Palladium (mg/kg)	Max. 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Nickel (mg/kg)	Max. 3.0	< 0.1	0.2	0.2	0.3	0.3
Microbiological specifications						
Salmonella	Absent in 25 g	Complies	Complies	Complies	Complies	Complies
Aerobic mesophilic total count	Max. 500 CFU/g	< 10	< 10	< 10	< 10	< 10
Enterobacteriaceae	Absent in 10 g	Complies	Complies	Complies	Complies	Complies
Cronobacter (Enterobacter) sakazakii	Absent in 10 g	Complies	Complies	Complies	Complies	Complies
Listeria monocytogenes	Absent in 25 g	n/a	Complies	Complies	Complies	Complies
Bacillus cereus	Max. 50 CFU/g	n/a	< 10	< 10	< 10	< 10
Yeasts	Max. 10 CFU/g	Complies	Complies	Complies	Complies	Complies
Moulds	Max. 10 CFU/g	Complies	Complies	Complies	Complies	Complies
Residual endotoxins[(e)]	Max. 10 EU/mg[(e)]	< LOR[(c)]	< LOR[(c)]	< LOR[(c)]	< LOR[(c)]	< LOR[(c)]

CFU: colony forming units; EU: endotoxin units; HPLC: high-performance liquid chromatography; LOQ: limit of quantification; LOR: level of reporting; max., maximum; min.: minimum; n/a: not applicable; RT: retention time

(a): These specifications refer to the water-free compound.

(b): In the form of free acetic acid and/or sodium acetate.

(c): LOR = 0.01 EU/mg.

(d): Not applicable to specifications; batch L06112K was not spray dried.

(e): Specifications for residual proteins and endotoxins are included to allow control on any impurity stemming from potential raw materials produced from alternative production processes (i.e. fermentation) and to provide reassurance that 2'-FL produced through Glycom's method of manufacture is controlled for residual levels of proteins and endotoxins.

(f): LOR = 0.03 %

(g): LOQ = 0.0018 w/w %

The NFI is a white to off-white powder. In response to a comment from a Member State on the level of purity of 2′-FL, the applicant indicated that the specification is set at a minimum of 95 % owing to the standard deviation of the assay method (around 2 %), which depends upon the hygroscopicity of 2′-FL and the method used for detection (charged aerosol detection). Taking into account the results of the analyses of five batches (Table 2), the applicant considered that the limit of 95 % is justified.

Upon a request by EFSA for clarification of the impurities of the NFI, the applicant proposed to include additional specifications for the residual starting materials (D-lactose and L-fucose) and the other carbohydrate-type by-products, difucosyl-D-lactose isomers and 2′-O-fucosyl-D-lactulose, which are formed during the manufacturing process. Only one of the difucosyl-D-lactose isomers, 3,2′-*O*-difucosyllactose, is naturally present in human milk (Erney et al., 2001), whereas the 2′,6′-*O*-difucosyllactose has not been reported to be present in human milk so far. 2′-*O*-Fucosyl-D-lactulose is an isomer of 2′-FL, arising from the isomerisation of the terminal glucose moiety of 2′-FL to fructose.

The specifications for residual solvents are provided for single and combined solvents, with maximum limits of 50 mg/kg and 200 mg/kg, respectively. The applicant confirmed that all solvents used in the process are used as processing aids. The applicant clarified that toluene, which is not used as a solvent during production, is formed as a volatile product of the hydrogenation reaction of benzyl-2′-FL and is removed during the spray drying step (Section 3.2). The applicant indicated that toluene is controlled by the internal specifications. Taking into consideration Joint FAO/WHO Expert Committee on Food Additives (JECFA) and European Medicines Agency (EMA) guidance for pharmaceuticals on residual solvents (ICH, 2011; EMA, 2014), the Panel considers that residual solvents do not raise safety concerns.

The protein content is specified at a maximum level of 0.01 %. Batch analyses indicate that protein content is below the limit of quantification (i.e. 0.0018 w/w %).

In response to a comment from a Member State, the applicant indicated that Palladium residue is maintained at levels below 0.1 mg/kg, as confirmed by the results of the batch analyses (Table 2). Nickel is also included in the product specifications (< 3.0 mg/kg), but at levels much lower than the Permitted Daily Exposure levels recommended by EMA for medicinal products. The potential presence of other metals is controlled by internal specifications.

Microbiological contaminants are also controlled by specifications. In response to a comment from a Member State, the applicant indicated that the procedures applied to the NFI are compliant with the hazard analysis and critical control points (HACCP), which are described in Regulation 2073/2005.

In response to a comment from a Member State, the applicant indicated that analytical measurements were performed using internationally recognised methods in accredited external laboratories. As no internationally recognised methods exist for the analysis of 2′-FL or 2′-FL-derived carbohydrates, methods were developed by the applicant and validated under Good Laboratory Practices (GLP).

In addition to the product specifications reported in Table 1, the applicant provided further internal product specifications, which have been claimed confidential. The internal product specifications include maximum limits for raw materials, intermediate products and processing aids used in the production process.

The Panel considers that the information provided on the composition, specification and data from batch testing do not raise safety concerns.

3.1.1. Stability of the NFI

A 6-month accelerated stability study (40 °C, 75 % relative humidity) has been performed on crystalline 2′-FL obtained from a single batch. Samples for chemical and microbiological analyses were packed into polyethylene bags (primary packaging material), and polyethylene/aluminium/polyester triple layer foil bags (secondary packaging material). 2′-FL was analysed by HPLC and water content was analysed by Karl Fischer titration.

The low water content after six months of storage (0.1 %) indicates that the packaging is appropriate for protecting 2′-FL from water absorption. Lactose, the potential degradation product of 2′-FL, was below 0.1 % after three and six months of storage. No unknown degradation products were observed

in the HPLC chromatogram. These results indicate that 2'-FL is stable and does not degrade when stored under accelerated conditions for periods of up to six months. Furthermore, microbiological purity was maintained throughout the duration of the 6-month study.

A 5-year long-term stability study (25 °C, 60 % relative humidity) on one batch of crystalline 2'-FL was on-going at time of the submission of this dossier. The applicant provided the analytical results obtained up to the 36-month point of this long-term stability study for samples of 2'-FL that were packaged in the same way as in the previous study.

The applicant indicated that no significant changes were observed in the value for 2'-FL for up to 36 months of storage. An increase in water content was observed by 18 months of storage (0.5 % vs. 0.1 % at time 0), which was maintained throughout the 36 months of storage (0.5 % at 24 months and 0.4 % at 36 months). Lactose, the potential degradation product of 2'-FL, was below 0.1 % at all time-points up to 36 months of storage. No unknown degradation products were observed in the HPLC chromatogram at any time point during the 36 months of storage. These results indicate that crystalline 2'-FL is stable and does not degrade when stored at a temperature of 25 °C for periods of up to 36 months. Microbiological purity was maintained throughout the duration of the 36 months.

Based on the available data, the shelf-life of crystalline 2'-FL, as proposed by the applicant, is 36 months when stored at room temperature protected from humidity.

The Panel notes that the stability experiments have been performed on a 'crystalline' product which contains 0.1 % water, whereas the NFI is a 'powder' with a water content of up to 9 %. However, despite this limitation, the Panel considers that, if protected by adequate packaging, the NFI is sufficiently stable.

3.1.2. Stability under the intended conditions of use

The NFI is intended to be used as an ingredient in IF, FOF, baby foods and a range of food products (Section 3.3). The applicant assessed the stability of the NFI added to IF (stored for three years at temperatures of 4, 20, 30 and 37 °C), yoghurt (stored for 21 days at 4 °C), citrus juice (stored for 28 days at 4 °C) and ready-to-drink flavoured milk (stored for 14 days (pasteurised) or 28 days (ultra-high temperature (UHT)-treated) at 4 °C). 2'-FL was added during the production process of these foods, to assess the effects of these processing on the NFI, as well as the stability of the NFI in storage. No significant losses of 2'-FL were observed in any of the conditions tested, which included pasteurisation and UHT treatments.

The Panel considers that the data provide sufficient information with respect to the stability of the NFI.

3.2. Effect of the production process applied to the NFI

2'-FL is produced by chemical synthesis through a two-stage manufacturing process.

In the first stage, the source raw materials L-fucose and D-lactose are used to produce the intermediate benzyl-2'-FL. D-Lactose complies with the specifications set in the European Pharmacopeia, whereas L-fucose is produced by the applicant through a controlled process. L-Fucose and D-lactose are derivatised and protected to give the stereo- and regio-selective α -(1→2)-glycosylation reaction. Benzyl-2'-FL is a high-quality and high-purity material, which is manufactured through a controlled process where all batches of benzyl-2'-FL are controlled for conformity to the specifications.

In the second stage, the intermediate benzyl-2'-FL is used to produce the final powdered product 2'-FL through a series of steps which include hydrogenation, purification and spray drying.

2'-FL is manufactured in compliance with good manufacturing practice. The applicant indicates that specifications are set according to HACCP principles, as defined by Regulation (EC) No 852/2004 and conformity is monitored by certificates of analysis at each key stage of the production process.

The Panel considers that the production process is sufficiently described and does not raise safety concerns.

3.3. Anticipated intake/extent of use of the NFI

3.3.1. Uses for infants and young children

The applicant intends to market 2'-FL as an ingredient in IF and FOF, including formulae for special medical purposes and other foods for infants and young children (Table 3). The proposed maximum use levels in all other food categories are based on the applicant's consideration of providing a similar intake to that of 2'-FL from mature breast milk on a mg/kg body weight basis.

Table 3: Proposed food uses for 2'-FL for infants and young children

Food category name	Proposed maximum use level
Foods for infants and young children	
Infant formulae as defined by Commission Directive 2006/141/EC[4]	2.4 g/L of reconstituted formula
Follow-on formulae as defined by Directive 2006/141/EC[4]	2.4 g/L of reconstituted formula
Processed cereal-based foods and baby foods for infants and young children as defined by Commission Directive 2006/125/EC[5]	1.2 g/100 g for solid foods
Other foods for young children	1.2 g/100 g for solid foods 1.2 g/L for beverages as consumed (2.4 g/L for young-child formula)
Dietary foods for infants and young children for special medical purposes as defined by Commission Directive 1999/21/EC[6] and special formulae for infants	As specified on a case-by-case basis in accordance with Commission Directive 1999/21

For infants aged 0 to 6 months, the estimated intake of 2'-FL was calculated from the use of 2 -FL in IF alone, under the assumption that it would be the only source of 2'-FL in this population group. The daily intake of liquid IF has been estimated to be 1 060 mL/day for infants aged 0 to 6 months, based on food consumption data used by EFSA (EFSA AFC Panel, 2006). This food intake scenario used by EFSA was based on a 3-month-old infant weighing 6.1 kg consuming 174 mL/kg body weight per day of IF at the 95th percentile. Assuming an intended use level of 2.4 g 2'-FL/L formula, the 95th percentile daily intake of 2'-FL from its use in IF alone is estimated to be 2.5 g/day (418 mg/kg body weight per day) in infants aged 0 to 6 months.

For infants aged 4 to 17 months, the applicant estimated intakes of 2'-FL from the consumption of IF and other infant-specific foods and beverages based on data available from the recent UK Diet and Nutrition Survey on Infants and Young Children (DNSIYC) conducted in 2011 (NatCen Social Research et al., 2013). Food consumption was estimated through a 4-day dietary diary. Intake estimates of 2'-FL were calculated based on the proposed use levels expressed in Table 3 for the category 'Foods for Infants and Young children'. These intakes are based on the intake of infant specific foods only for this food category. The percentage of users of foods from this category was high among all age groups evaluated in the current intake assessment (above 84.7 %), with the highest percentage users found among infants aged 4 to 6 months (98.1 %) Large user percentages within a population group typically lead to similar results for the all-person and all-user consumption estimates. Consequently, only the all-user intake results will be discussed in detail.

It was determined that infants aged 4 to 6 months have the greatest mean all-user intakes of 2'-FL on an absolute basis of 2.75 g per day, while infants aged 7 to 12 months had the highest 95th percentile all-user intakes at 6.12 g per day, respectively (Table 4). Young children aged 13 to 17 months had the lowest mean and 95th percentile all-user intakes at 1.28 and 3.80 g/day, respectively.

[4] Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC. OJ L 401, 30.12.2006, p. 1–33.
[5] Commission Directive 2006/125/EC of 5 December 2006 on processed cereal-based foods and baby foods for infants and young children. OJ L 339, 6.12.2006, p. 16–35.
[6] Commission Directive 1999/21/EC of 25 March 1999 on dietary foods for special medical purposes. OJ L 91, 7.4.1999, p. 29–36.

On a body weight basis, infants aged 4 to 6 months were identified as having the highest mean and 95th percentile all-user intakes of all groups at 338 and 668 mg/kg body weight per day, respectively. Young children aged 13 to 17 months had the lowest mean and 95th percentile all-user intakes at 118 and 355 mg/kg body weight per day, respectively (Table 5).

In response to a comment from a Member State on the reason behind the use of this NFI, the applicant indicated that the NFI is identical to the 2'-FL that is present in human breast milk.

Table 4: Summary of the estimated daily intake of 2'-FL from all proposed food categories[(a)] in the UK by population group, based on data from DNSIYC 2011

Population group	Age group (months)	Total (n)	All-person consumption (g/day)		All-user consumption (g/day)			
			Mean	95th percentile	%	n	Mean	95th percentile
Infants	4–6	329	2.70	5.45	98.1	323	2.75	5.49
Infants	7–12	1 319	2.44	5.98	94.8	1 252	2.58	6.12
Young children	13–17	1 035	0.87	3.34	67.5	688	1.28	3.80
Total	4–17	2 683	1.87	5.24	84.7	2 263	2.21	5.50

(a): For the DNSIYC, only intakes based on the category 'Foods for infants and young children' were included in the assessment.

Table 5: Summary of the estimated daily per kg body weight intake of 2'-FL from all proposed food categories[(a)] in the UK by population group, based on data from DNSIYC 2011

Population group	Age group (years)	Total (n)	All-person consumption (mg/kg body weight per day)		All-user consumption (mg/kg body weight per day)			
			Mean	95th percentile	%	n	Mean	95th percentile
Infants	4–6	329	332	666	98.1	323	338	668
Infants	7–12	1 319	259	636	94.8	1 252	273	641
Young children	13–17	1 035	79	316	67.5	688	118	355
Total	4–17	2 683	199	580	84.7	2 263	234	601

(a): For the DNSIYC, only intakes based on category 'Foods for infants and young children' were included in the assessment.

3.3.2. Other food uses for other population groups

The applicant intends to market 2'-FL as an ingredient in a variety of food products including food supplements (Table 6). In a tiered approach the applicant first estimated intakes of the NFI by using the EFSA Food Additive Intake Model (FAIM) tool which is based on summary statistics of consumption data of the EFSA Comprehensive Food Consumption Database (EFSA, 2012). For a refined intake estimate, the applicant used individual consumption data recorded by the UK National Diet and Nutrition Survey (NDNS) for the years 2008–2010 (UKDA, 2012).

The proposed maximum use levels of 2'-FL in all food categories are based on the applicant's consideration of providing similar intakes to those of 2'-FL from breast milk for infants on a mg/kg body weight basis.

Table 6: Uses and use levels for 2'-FL (g/kg)[a] proposed by the applicant

UK NDNS food categories	Food classification system used by the FAIM Tool	Food category name	Suggested serving size	Maximum level per serving	Proposed maximum use level (g/kg or g/L)[b]
Dairy products and analogues:	1.1	Unflavoured pasteurised and sterilised (including UHT) milk	200 g	1.2 g/L	1.2
Unflavoured pasteurised, sterilised milk and milk–recipes,	1.2	Unflavoured fermented milk products	125 g	1.2 g/L for beverages. Other foods: 1.2–2.4 g/serving	1.2 g/L for beverages 9.6–19.2 g/kg for solids
	1.3	Unflavoured fermented milk products, heat-treated after fermentation			
	1.4	Flavoured fermented milk products including heat-treated products			
Unflavoured fermented milk products, Flavoured fermented milk products, Dairy analogues.	1.8	Dairy analogues, including beverage whiteners	200 g	1.2 g/L for beverages 1.2–2.4 g/serving for other foods	1.2 g/L for beverages 6–12g /kg for solids
			3 g	1.2 g per sachet whitener	400
Cereal bars	7.2	Fine bakery wares. Cereal bars only	n/a[c]	1.2 g/100 g	12
Table top sweeteners	11.4	Table top sweeteners	6 g sachet	1.2 g per sachet	200
Infant formula and follow-on formula. Foods for infants and young children, Dietary foods (including powders) for weight control diets, Foods suitable for people intolerant to gluten.	13.1.1 [d]	Infant formulae as defined by Commission Directive 2006/141/EC[7(e)]	n/a[d]	2.4 g/L of reconstituted formula	2.4
	13.1.2 [d]	Follow-on formulae as defined by Directive 2006/141/EC[7(e)]	n/a[d]	2.4 g/L of reconstituted formula	2.4
	13.1.3 [d]	Processed cereal-based foods and baby foods for infants and young children as defined by Commission Directive 2006/125/EC[8(e)]	n/a[c]	1.2 g/100 g for solid foods	12
	13.1.4 [d]	Other foods for young children[e]	n/a[c]	1.2 g/100 g for solid foods	12
			n/a[c]	1.2 g/L for beverages as consumed 2.4 g/L for young-child formula	1.2 for beverages 2.4 for young-child formula
	13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC[9]	Case-by-case basis	As specified on a case-by-case basis in accordance	Case-by-case basis

[7] Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC. OJ L 401, 30.12.2006, p. 1-33.
[8] Commission Directive 2006/125/EC of 5 December 2006 on processed cereal-based foods and baby foods for infants and young children. OJ L 339, 6.12.2006, p. 16-35.

UK NDNS food categories	Food classification system used by the FAIM Tool	Food category name	Suggested serving size	Maximum level per serving	Proposed maximum use level (g/kg or g/L)[(b)]
		(excluding products from food category 13.1.5 'Dietary foods for infants and young children for special medical purposes and special formulae for infants')[(f)]		with Commission Directive 1999/21	
	13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)	250 g drinks 30 g bars	1.2 g/meal replacement	4.8 for drinks 40 for bars
	13.4	Foods suitable for people intolerant to gluten as defined by Commission Regulation (EC) No 41/2009[10]	Bread products: 40 g Pastas: 55 (unprepared) to 110 g (prepared)	1.2–2.4 g/serving	11–60
Beverages: Fruit & vegetable juices and nectars, Flavoured drinks, Coffee, tea (except black tea), Herbal and fruit infusions.	14.1.2	Fruit juices as defined by Directive 2001/112/EC[11] and vegetable juices	n/a[(c)]	1.2 g/L	1.2
	14.1.3	Fruit nectars as defined by Directive 2001/112/EC[11] and vegetable nectars and similar products	n/a[(c)]	1.2 g/L	1.2
	14.1.4	Flavoured drinks	n/a[(c)]	1.2 g/L	1.2
	14.1.5	Coffee, tea (excluding black tea), herbal and fruit infusions, chicory; herbal and fruit infusions and chicory extracts; tea, plant, fruit and cereal preparations for infusions, as well as mixes and instant mixes of these products	250	1.2–2.4 g/serving	4.8–9.6
Food supplements as defined in Directive 2002/46/EC[12]	17.1	Food supplements supplied in a solid form including capsules and tablets and similar forms, excluding chewable forms	n/a[(c)]	3.0 g per day	Maximum intake of 3.0 g/day[(g)]

[9] Commission Directive 1999/21/EC of 25 March 1999 on dietary foods for special medical purposes. OJ L 91, 7.4.1999, p. 29–36.
[10] Commission Regulation (EC) No 41/2009 of 20 January 2009 concerning the composition and labelling of foodstuffs suitable for people intolerant to gluten. OJ L 16, 21.1.2009, p. 3–5.
[11] Council Directive 2001/112/EC of 20 December 2001 relating to fruit juices and certain similar products intended for human consumption. OJ L 10, 12.1.2003, p. 58–66.
[12] Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to dietary supplements. OJ L 183, 12.7.2002, p. 51–57.

UK NDNS food categories	Food classification system used by the FAIM Tool	Food category name	Suggested serving size	Maximum level per serving	Proposed maximum use level (g/kg or g/L)[b]
	17.2	Food supplements supplied in a liquid form	n/a[c]		
	17.3	Food supplements supplied in a syrup-type or chewable form	n/a[c]		

EC: European Commission; EU: European Union; n/a: not applicable

(a): While it is proposed that 2-FL will be added to these food categories, including 'Dairy products and analogues', once placed on the market, all food products with added 2-FL would be appropriately reclassified and labelled .

(b): The proposed maximum use level is presented on a g/kg basis for solids and on a g/L basis for liquids.

(c): A serving size is not provided on the basis that the use level is specified on a per L/kg basis and not on a per serving basis.

(d): The suggested serving size for infant formula is according to the manufacturer's instructions and according to the baby's age and weight.

(e): These categories were also assessed using food consumption data from the DNSIYC.

(f): This category was not included in the UK NDNS intake assessment as these products should be assessed on a case-by-case basis, and are not widely consumed by the general population.

(g): Food supplements were not included in the combined intake assessment as a maximum of 3.0 g of 2-FL per day through a single supplement dose would be recommended.

Mean and high-intake estimates derived with the EFSA FAIM tool are presented in Table 7. A total of 26 different dietary surveys carried out in 17 different European countries are included in the FAIM tool. Mean intake values for the total population are calculated per age group by summing up the mean exposures from all contributing food sources in a given survey. High-level values of a given food category are calculated either as the 95th percentile of consumers-only, when the number of consumers is ≥ 60, or as the mean of consumers only, when the number of consumers is < 60.

The applicant used the lower and upper ends of the proposed use level ranges per food category for the calculation in the FAIM tool. The food categories included in the calculations were dairy products and analogues, bakery wares, sugars, syrups, honey and table-top sweeteners, foods intended for particular nutritional uses as defined by Directive 2009/39/EC[13] and non-alcoholic beverages, as per the proposed uses. The results are presented in Table 7. The mean intakes of 2'-FL in the total population, as reported in the FAIM tool, ranged from 24 to 60 mg/kg body weight per day in adolescents up to 96 to 204 mg/kg body weight per day in toddlers when the lower use levels were used, and ranged from 30 to 95 mg/kg body weight per day in adolescents and up to 320 to 666 mg/kg body weight per day in toddlers when the maximum use levels were used (Table 7). High-level intakes ranged from 44 to 130 mg/kg body weight per day in adolescents and up to 213 to 452 mg/kg body weight per day in toddlers when the lower use levels were used, and ranged from 89 to 286 mg/kg body weight per day in the elderly up to 822 to 1,928 mg/kg body weight per day in toddlers when the maximum use levels were used.

The Panel notes that the EFSA FAIM tool has been developed as a screening tool for deriving chronic intake estimates for food additives for different population groups throughout several European countries. The tool is based on summary statistics of the EFSA Comprehensive database. The FoodEx classification used by this tool has been linked mainly to the level 2, and for few foods up to level 3, of the Food Classification System (FCS) as presented in Commission Regulation (EU) N° 1129/2011[14]. Intake levels from all contributing food sources are obtained by adding the high-level (usually the 95th percentile) of exposure from the food category providing the highest estimate plus the estimated mean exposure values for the remaining categories. The underlying methodology of the FAIM tool generally leads to an overestimation of the estimated intakes as expressed by the EFSA instructions for the use of the FAIM tool (EFSA, 2012).

Table 7: Summary of estimated intakes of 2'-FL from proposed foods uses using the EFSA FAIM tool

Population	Ages	Mean intakes (mg/kg body weight per day)		High level intakes (mg/kg body weight per day)	
		Lower use level	Maximum use level	Lower use level	Maximum use level
Toddlers	12 to 35 months	96–204	320–666	213–452	822–1928
Children	3 to 9 years	53–153	75–263	104–363	172–682
Adolescents	10 to 17 years	24–60	30–95	44–130	68–362
Adults	18 to 64 years	22–87	35–152	51–163	90–304
Elderly	65 years and older	19–83	30–158	36–147	89–286

For the refined intake estimate based on UK individual consumption data, individual food codes representative of each proposed food use were taken from the food code list associated with the UK NDNS food consumption survey and grouped according to the proposed uses for the LNnT assessment presented in Table 6. The daily intake of 2'-FL was calculated at individual level, and represents projected 4-day averages for each individual from days 1 to 4 of NDNS data, in the UK population. High percentile intake estimates were derived from the distribution of these average amounts. Results are provided in Tables 8 and 9.

In relation to all-user intakes, toddlers had the highest mean and 95th percentile intake of about 1.67 g per day, whereas women of child-bearing age had the highest intakes at the 95th percentile of about 5 g per day (Table 8). On a body weight basis, toddlers were identified as having the highest

[13] European Parliament and the Council of the European Union. Directive 2009/39/EC of the European Parliament and of the Council of 6 May 2009 on foodstuffs intended for particular nutritional uses. OJ L 124, 20.05.2009, p. 21–29.

[14] Commission Regulation (EU) No 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives. OJ L 295, 11.11.2011, p.1–177.

mean and 95th percentile intake of any population group of 120 and 247 mg/kg body weight per day, respectively (Table 9).

Table 8: Summary of the estimated daily intake of 2'-FL from proposed food categories by different population groups in the UK, per person, based on data from NDNS

Population group	Age group (years)	All-person consumption (g/day)		All-user consumption (g/day)			
		Mean	95th percentile	%	n	Mean	95th percentile
Toddlers	1–3	1.67	3.41	100	219	1.67	3.41
Children	4–10	1.43	3.12	100	423	1.43	3.12
Teenagers	11–18	1.31	3.13	99.7	451	1.31	3.14
Women of child-bearing age	19–40	1.61	4.98	100	216	1.61	4.98
Female adults	19–64	1.63	4.87	99.9	460	1.63	4.87
Male adults	19–64	1.36	4.01	99.6	344	1.36	4.01
Elderly adults	65 years and older	1.35	3.60	99.8	223	1.35	3.61

Table 9: Summary of the estimated daily per kilogram body weight intake of 2'-FL from proposed food categories by different population groups in the UK, based on data from NDNS

Population group	Age group (years)	All-person consumption (mg/kg body weight per day)		All-user consumption (mg/kg body weight per day)			
		Mean	95th percentile	%	n	Mean	95th percentile
Toddlers	1–3	120	247	100	219	120	247
Children	4–10	58	135	100	423	58	135
Teenagers	11–18	23	55	99.7	451	23	55
Women of child-bearing age	19–40	24	75	100	216	24	75
Female adults	19–64	24	74	99.9	460	28	74
Male adults	19–64	16	50	99.6	344	16	50
Elderly adults	65 years and older	18	55	99.8	223	18	55

One Member State noted that the intake estimates calculated by the applicant do not include the potential additional intake of 2'-FL from food supplements, as proposed in the application. According to the applicant, the most significant target group for food supplements will be women of child-bearing age. Considering the highest estimated 95th percentile daily intake of 2'-FL at 5 g for women of child-bearing age plus the maximum intended daily intake from food supplements of 3 g, 2'-FL intakes in this population group could reach up to 8 g per day.

In response to another comments from Member States, the applicant indicated that the proposed use levels of 2'-FL would result to intakes which are within the range of human milk on a per kg body weight basis. Table 5 shows that the high-intake scenario for infants would be about 670 mg/kg body weight per day, which is a level within the range of the estimated intake from breast milk. Table 9 shows the highest 95th percentile intake for toddlers (247 mg/kg body weight per day), whereas for older age groups the estimates are lower. In his response the applicant also noted that the estimated daily intakes of 2'FL for older age groups also stay below the well-known levels of other non-digestible carbohydrates that can occasionally cause transient tolerability effects in older age groups (e.g. lactose (Mattar et al., 2012; Heaney, 2013), lactulose (Havenaar and Van Dokkum, 2001), inulin (Coussement and Franck, 2001), oligofructose (Coussement and Franck, 2001; Bali et al., 2015), and galacto-oligosaccharides (Torres et al., 2010)).

The Panel considers that the refined intake estimate based on UK individual consumption data is sufficiently conservative, as it is based on the assumption that all proposed food items consumed by an individual actually contain the NFI at the maximum specified level of use.

3.4. Information from previous exposure to the NFI or its source

Human milk contains as its third largest solid component a fraction of oligosaccharides (Kunz and Rudloff, 1993; Bode, 2012; Newburg, 2013), which are known as human milk oligosaccharides (HMOs).

2′-FL is one of the naturally occurring fucosylated oligosaccharides that are present in human milk (Erney et al., 2000; Chaturvedi et al., 2001a; Musumeci et al., 2006; Asakuma et al., 2008; Castanys-Muñoz et al., 2013). However, 2′-FL is not detected in the milk of women (approximately 20 % of women) who do not express a specific enzyme, α-1,2-fucosyltransferase, in their mammary glands, which is responsible for fucosylating lactose at the 2′-O-position. This phenotype is referred to as the 'non-secretor´ phenotype and forms, together with the phenotype for another enzyme (α-1,4-fucosyltransferase, required to synthesise the so-called 'Lewis' antigens a and b), the basis for categorisation of human milk into four different phenotypes (Thurl et al., 1997, 2010; Coppa et al., 2011).

The applicant provided several publications on the 2′-FL content in human milk in relation to secretor- and Lewis-blood group status (Thurl et al., 1997; 2010, Coppa et al., 2011; Galeotti et al., 2012, 2014), ethnicity (Erney et al., 2000; Musumeci et al., 2006), lactation period (Coppa et al., 1999; Erney et al., 2000; Sumiyoshi et al., 2003; Asakuma et al., 2008; Leo et al., 2010; Bao et al., 2013), preterm birth (Nakhla et al., 1999; Gabrielli et al., 2011), and in mature milk (Chaturvedi et al., 1997, 2001a; Erney et al., 2000, 2001; Sumiyoshi et al., 2003; Morrow et al., 2004; Leo et al., 2010; Thurl et al., 2010; Asakuma et al., 2011; Coppa et al., 2011; Galeotti et al., 2012; Smilowitz et al., 2013; Hong et al., 2014). The concentration of 2′-FL in breast milk appears to depend on the lactation period, with higher levels reported in colostrum and decreased levels observed thereafter (Erney et al., 2000). Although the levels of 2′-FL decrease as lactation progresses, the levels of 2′-FL remain above 1 500 mg/L at 31 to 452 days post-partum (Coppa et al., 1999; Erney et al., 2000; Thurl et al., 2010).

According to the applicant the content of 2′-FL, from pooled data from different phenotypes milks, ranged between 1 100 and 4 260 mg/L in mature breast milk (Bao et al., 2013; Galeotti et al., 2014), with an average of approximately 2 350 mg/L. However, concentrations around 600 mg/L have also been reported (Sumiyoshi et al., 2003; Leo et al. 2010). Concentrations of up to 7 000 mg/L have been reported in the milk from secretor phenotype mothers (Galeotti et al., 2012; Gabrielli et al., 2011).

The applicant indicated that the content of 2′-FL in colostrum ranged from 1 120 to 4 900 mg/L (Musumeci et al., 2006; Bao et al., 2013); however, concentrations of 8 400 mg/L have also been reported from secretor phenotype mothers (Musumeci et al., 2006).

Based on the levels of 2′-FL in mature breast milk and on a 6.5 kg infant drinking approximately 1 L of breast milk per day (Davies et al., 1994, Hester et al., 2012), the applicant indicated that the average intake of 2′-FL is approximately 170 to 660 mg/kg body weight per day. However, in infants from secretor mothers, the intake of 2′-FL from mature breast milk may be up to approximately 1 150 mg/kg body weight per day.

According to the applicant, for newborn infants, the average intake of 2′-FL from colostrum is approximately 80 to 360 mg/kg body weight per day based on a 3.4 kg newborn infant (WHO, 2014) drinking an average of 250 mL of breast milk per day during the first 5 days (Hester et al., 2012). However, in newborns from secretor mothers, the intake of 2′-FL from colostrum may be up to approximately 620 mg/kg body weight per day.

2′-FL has been also detected in domestic farm animal milk, such as cow's, sheep's, camel's, pig's and goat's milk (Urashima et al., 1994; Tao et al., 2010; Aldredge et al., 2013; Albrecht et al., 2014); however, data on the levels of 2′-FL in animal milk have not been reported. The amount of total oligosaccharides in domestic farm animal milks has been reported to be lower than those in human milk (Tao et al., 2009; Meyrand et al., 2013).

The Panel notes that the history of human exposure to 2′-FL is limited primarily to that of breast-fed infants. The Panel also notes that the proposed maximum intake level of the NFI by infants is within the range of 2′-FL levels found in mature breast milk, as reported in the literature, although at the high end of this range.

3.5. Nutritional information on the NFI

2′-FL is, on average, the most abundant HMO in breast milk in the general population of breastfeeding women. However, approximately 20 % of breastfeeding women lack the enzyme in their mammary glands that is responsible for the biosynthesis of 2′-FL (referred to as non-secretors), and, thus, their milk is devoid of 2′-FL (and other 2′-O-fucosylated oligosaccharides). The majority (80 %) of breastfeeding women, however, produce milk with such a high level of 2′-FL that the substance remains the most abundant individual HMO when values obtained from different women with varying milk types are averaged or when samples from different women with varying milk types are pooled and an average obtained.

The applicant proposes the addition of 2′-FL to IF, in order to bring IF and FOF closer to the composition of breast milk. The applicant also proposes the addition of 2′-FL to other food categories, such as bakery wares, sugars, syrups, honey and table-top sweeteners, PARNUTS (foodstuffs for particular nutritional uses) and beverages.

The impact of uses other than IF would be comparable to the current use of fibre-type oligosaccharides (including laxative effects) which are added to a range of food products.

The Panel considers that consumption of the NFI is not nutritionally disadvantageous.

3.6. Microbiological information on the NFI

Microbiological limits, which also include microbial endotoxins, are presented in the product specifications (Table 1). The applicant provided analytical results for five batches which were compliant with the specifications (Table 2).

The Panel considers that the microbiological information provided does not raise safety concern.

3.7. Toxicological information on the NFI

3.7.1. Absorption, distribution, metabolism and excretion

HMOs, including 2′-FL, are reported to be resistant to hydrolysis by digestive enzymes in *in vitro* studies (Engfer et al., 2000; Gnoth et al., 2000).

HMO fermentation in the colon in infants who received a load of HMO (a purified oligosaccharide fraction from their mothers' milk) was determined through a breath hydrogen test. The observations from this test suggest that HMOs, consumed as a load, are fermented in the colon (Brand-Miller et al., 1995, 1998).

The pattern and the amount of HMO in the faeces of breast-fed infants were compared with the pattern and amount of HMO in ingested breast-milk (Chaturvedi et al., 2001b; Coppa et al., 2001). These studies report that 40–50 % and 97 %, respectively, of the ingested amount of HMO is excreted unchanged in the faeces.

Urinary excretion of HMO has been investigated in breast-fed infants. A bolus of ^{13}C-labelled glucose or ^{13}C-labelled galactose was given to lactating mothers in order to obtain ^{13}C-labelled HMO, which could then be detected in the breast-fed infants' urine (Obermeier et al., 1999; Rudloff et al., 2006, 2012; Rudloff and Kunz, 2012; Dotz et al., 2014). Another study compared the amount of oligosaccharides in the urine of breast-fed infants with the oligosaccharides in their mothers' milk (Chaturvedi et al., 2001b). These studies suggest that a small portion of HMO (approximately 1 to 2 % of the ingested amount) is excreted unchanged in infants' urine. Data from *in vitro* studies using the Caco-2 human intestinal epithelial cell model suggest that neutral HMOs (like 2′-FL) are transported across the intestinal epithelium by receptor-mediated transcytosis, as well as by paracellular pathways (Gnoth et al., 2001). In response to a comment from a Member State, the

applicant indicated that the use of 2′-FL in vulnerable individuals would occur under medical supervision, as required for foods for special medical purposes.

Overall, the data indicate that a large proportion of the ingested HMO, including 2′-FL, reaches the large intestine and is either fermented by the intestinal microbiota or excreted in the faeces.

There are no indications that the absorption of the NFI may differ from that of 2′-FL from breast milk, and the consumption levels of the NFI would likely be similar to those of 2′-FL from breast milk by infants.

The Panel considers that the limited amount of the NFI that could be absorbed, as suggested by the above data, does not raise safety concerns.

3.7.2. Genotoxicity

The applicant provided three studies on the potential mutagenicity of 2′-FL, with a purity of 99 % (Coulet et al., 2014) and 96.9 % (*in vitro* micronucleus assay study). These studies were conducted in compliance with the Organisation for Economic Co-operation and Development (OECD) principles of GLP and the appropriate OECD test guidelines.

In the bacterial reverse mutation assay (Ames test), five *Salmonella typhimurium* strains (TA98, TA100, TA1535, TA1537 and TA102) were exposed to 2′-FL, at levels up to 5 000 µg/plate, in the presence or absence of metabolic activation (S9-mix), using the plate-incorporation and pre-incubation methods (OECD test guideline No 471; OECD, 1997a) (Coulet et al., 2014). No signs of cytotoxicity or precipitation were observed in any strain treated with 2′-FL in the presence or absence of S9-mix. Exposure to 2′-FL resulted in few significant increases in the number of revertants, which however, were not dose-related. Overall, there were no biologically significant increases in the number of revertants compared with the negative control, at any concentration of 2′-FL, either in the presence or absence of S9-mix. This study showed no mutagenicity of 2′-FL, with and without S9-mix, up to the highest tested concentration of 5 000 µg/plate.

In the *in vitro* mammalian cell gene mutation test, L5178Y $Tk^{+/-}$ mouse lymphoma cells were incubated with 2′-FL for four hours, with or without S9-mix, and for 24 hours without S9-mix (Coulet et al., 2014). This study was conducted in compliance with the OECD test guideline No. 476 (OECD, 1997b). No signs of precipitation or cytotoxicity were observed in the cells exposed to any concentration of 2′-FL. No statistically significant increases in the frequency of mutations were observed in cells treated with 2′-FL, with or without S9-mix. This study showed no mutagenicity of 2′-FL, with or without S9-mix, up to the highest tested concentration of 5 000 µg/mL.

In response to comments from Member States, the applicant provided the study report on an *in vitro* micronucleus assay with 2′-FL (OECD test guideline No 487, OECD 2014) (unpublished study report, 2015a). 2′-FL was tested in human peripheral blood lymphocytes, with or without metabolic activation (S9-mix). The highest concentration tested of 2′-FL was 2 000 µg/mL. In the first experiment 2′-FL was tested for three hours with a 27-hour harvest time, with or without S9-mix, whereas in the second experiment, the exposure lasted 24 hours with a 24-hour harvest time, without S9-mix. 2′-FL did not induce a statistically significant increase in the number of mono- or binucleated cells with micronuclei, with or without S9-mix. This study showed neither clastogenicity nor aneugenicity of 2′-FL, with or without S9-mix, up to the highest tested concentrations of 2 000 µg/mL.

The Panel considers that the information provided does not raise safety concerns as regards the genotoxicity of the NFI.

3.7.3. Repeated exposure toxicity studies

The potential toxicity of the NFI was investigated in a sub-chronic (90-day) oral toxicity study in Wistar (Crl:WI(Han)) rats, which was preceded by a 14-day dose range-finding study (Coulet et al., 2014). In all studies, synthetically produced 2′-FL with a purity of 99 % was used as the test material.

Rat pups were used to adapt the standard OECD test guideline No 408 to the intended use of this NFI in infants (OECD, 1998a; Barrow, 2007). In both studies, fructo-oligosaccharides were given to a reference control group on the basis that they are used in IF.

In the non-GLP 14-day dose range-finding study with Wistar (Crl:WI(Han)) rats, 0 (vehicle control), 2 000, 5 000, or 7 500 mg/kg body weight per day of 2′-FL were administered, by gavage, from post-natal day (PND) 7 until PND 20 (weaning) (Coulet et al., 2014). Liquid faeces and/or yellowish liquid faeces, which were accompanied by erythema in the urogenital region, were observed in most of the animals in the mid- and high-dose 2′-FL groups. Between days 0 and three, lower body weight gains were reported in the mid- and high-dose 2′-FL groups than in the control groups. At the end of the study, body weights were not different between the test and control groups. Based on the results of this study, the authors determined that the highest dose of 2′-FL considered suitable for the main study in rats should be lower than 7 500 mg/kg body weight per day.

In the sub-chronic 90-day toxicity study with a 4-week recovery period, Wistar (Crl:WI(Han)) rat pups (PND 7) were administered, orally by gavage, 0 (water vehicle control), 2 000, 5 000, or 6 000 mg/kg body weight per day of 2′-FL for 90 consecutive days (Coulet et al., 2014). This study was conducted in compliance with the OECD principles of GLP and in accordance with OECD Test No. 408 (OECD, 1998a, b).

The cause of death of two animals (one male and one female in the high-dose 2′-FL group), which were found dead on day two, could not be determined by histopathological examination.

A few statistically significant changes in organ weights were reported at the end of the administration period in the 2′-FL groups compared with the control group: a decrease in absolute adrenal weights in males in the mid- and high-dose 2′-FL groups; a decrease in relative adrenal weights in 2′-FL high-dosed males; an increase in relative heart weight in males in the 2′-FL mid-dose group; and a decrease in absolute brain weight and relative kidney weights in 2′-FL high-dosed females.

Histopathological examination of the kidneys revealed a higher incidence of minimal cortical tubular epithelial cytoplasmic vacuolation in 2′-FL mid-dosed (6 out of 10) and high- dosed (3 out of 9) females at the end of the administration period than in the control group (1 out of 10). This could be associated with the decrease in relative kidney weight noted in females in the high-dose 2′-FL group. No cortical tubular epithelial cytoplasmic vacuolation was observed in males at the end of the administration period. In the recovery groups, minimal cortical tubular epithelial cytoplasmic vacuolation was seen in the 2′-FL high-dosed females (3 out of 5) and in the female control group (3 out of 5). No other compound-related histopathological findings were reported.

A significant decrease in red blood cells, haemoglobin and packed cell volume were observed in female rats in the mid- and high-dose 2′-FL groups, compared with controls. A significant decrease in serum sodium levels was observed in males and females in the mid- and high-dose 2′-FL groups, compared with controls. A significant decrease in serum bilirubin was observed in the 2′-FL high-dosed males compared with controls, whereas a significant decrease in protein and globulin was observed in the 2′-FL mid- and high-dosed males compared with the controls. A slight increase in creatinine was observed in females in the mid- and high-dose 2′-FL groups compared with the control group.

Based on the decrease in the relative kidney weight in the 2′-FL high-dosed female group, two unexplained deaths in the high-dose 2′-FL group and high-dosed female group, and the significant changes in the haematological and clinical blood parameters in the 2′-FL mid- and high-dosed group, the Panel considers that the no observed adverse effect level (NOAEL) is 2 000 mg/kg body weight per day.

3.7.4. Human studies

One Member State indicated the absence of studies on the safety of the NFI in infants aged 0-6 months. In response to this comment and to a request by EFSA, the applicant provided an unpublished study report on a double-blind, randomised, controlled clinical trial on the effect of a combination of two oligosaccharides, 2′-FL and lacto-*N*-neotetraose (LNnT), in infants (unpublished study report, 2015b, d). The Panel notes that LNnT is subject to another application as a NFI which has been assessed in parallel with the safety assessment of 2′-FL (EFSA NDA Panel, 2015). A total of 175 healthy, full-term infants (aged 0–14 days) were randomised to consume either a formula with added LNnT and 2′-FL (n = 88; 1.0–1.2 g/L of 2′-FL and 0.5–0.6 g/L of LNnT for reconstituted formula) or the same formula without oligosaccharides (n = 87) up to six months of age. The formula used was a standard whey-predominant starter IF with long-chain polyunsaturated fatty acids and without oligosaccharides (66.9 kcal/100 mL reconstituted formula, 1.9 g protein/100 kcal powder with

a whey:casein ratio of 2.5:1 (71.6 % whey and 28.4 % casein)). Exclusively breast-fed (BF) infants (beginning at three months of age) were included as a reference group.

This study aimed to demonstrate a non-inferiority in weight gain between the two formula-fed groups up to four months of age (primary outcome). Differences in body weight, body length, head circumference, 'digestive tolerance', stool characteristics, behaviour patterns, formula intake, use of concomitant medications and adverse events between the two formula-fed groups were also investigated (secondary outcomes).

The study showed that the weight gain in the test group was non-inferior to the weight gain in the control group in both the intention-to-treat (ITT) and per-protocol (PP) populations (n = 71 in the test formula group and n = 75 in the control formula group) (in the ITT population, −0.13 g/day (two-sided 95 % confidence interval (CI) −1.63 to 1.37; p = 0.864); in the PP population, −0.30 g/day (two-sided 95 % CI −1.94 to 1.34; p = 0.715)).

The mean weight, length, head circumference and BMI of infants aged 0–4 months were comparable with the corresponding World Health Organization (WHO) standard growth curves. No differences in mean weight, length, head circumference or BMI between groups were observed at any monthly visits (except for lengths at the 3-month visit).

Stool consistency scores on the Bristol Stool scale were not significantly different between the test and control formula-fed groups, except for scores for 2-month-old infants in the test formula group, who had higher scores (i.e. softer stools) than infants who consumed the control formula. No significant difference was observed in the number of stools reported by parents between the two formula-fed groups.

Overall, adverse events were not significantly different between the test and control formula-fed groups except for the incidence of bronchitis, which was significantly lower in infants in the intervention group than in those in the control group (odds ratio (OR) = 0.30; 95 % CI 0.11–0.73; p = 0.004). Antibiotic use was significantly lower in infants in the intervention group than in the control group (25.0 % vs. 41.4 %; OR = 0.47; 95 % CI 0.23–0.94, p = 0.025).

Microbiota composition and the presence of pathogens in stools from infants of three months of age were reported. The alpha diversity of the microbiota of the BF group was significantly lower than the diversity of both formula groups, but the diversity of the test group was significantly reduced compared with the diversity of the control formula group and, therefore, closer to the BF group. An increase of *Bifidobacterium,* a decrease in *Escherichia* and unclassified *Peptostreptococcaceae* were reported for infants in the test formula group compared with the infants in the control formula group, showing proportions closer to the BF group. The number of infants with at least one detectable viral, bacterial or eukaryotic (protista) pathogen in stool samples was not statistically different between the two formula-fed groups.

The Panel notes that this study showed no difference in growth in infants who consumed a formula added with the combination of 2'-FL and LNnT (at the concentrations tested in the study), compared with the control formula infants, and that the growth curves were comparable to the WHO standard curves. The Panel also notes that the results on stool and microbiota endpoints do not raise safety concerns.

The Panel also notes that the concentration of the NFI in the infant formula studied in this trial, was only about half of the maximum level as proposed by the applicant.

The applicant provided an unpublished study report on a placebo-controlled, double-blind, parallel, dose-response trial in 100 healthy adults (49 females, age range: 19–57 years) who were randomised into 10 groups (n = 10) to consume once daily either 2'-FL or LNnT alone (5, 10, 20 g of LNnT or 2'-FL), or a combination of 2'-FL and LNnT (5, 10, 20 g as the total amount of the combination of 2'-FL and LNnT at a ratio of 2:1), or placebo (glucose) for two weeks (unpublished study report, 2015c). At the end of the intervention, a significant increase in nausea, rumbling, bloating, passing gas, diarrhoea, loose stools and urgency was reported for participants who consumed 20 g of 2'-FL compared with the placebo group. No difference to the placebo group was observed in the group with an intake of 5 and 10 g 2'-FL per day. At the end of the intervention, a significant increase in passing gas was reported for participants who consumed either 20 g or 10 g of LNnT, compared with the placebo group. Stool consistency scores on the Bristol Stool scale were not significantly different

between the interventions and placebo groups. In total, 44 participants reported adverse events, which could cover more than one symptom, and a total of 78 symptoms were recorded. Gas/flatulence was the most commonly reported adverse event, which was followed by stomach pain, diarrhoea and rumbling. A higher incidence of adverse events was observed in participants who consumed the single oligosaccharide at the highest dose tested (20 g). All adverse events were reported to be 'mild' and no serious adverse events were reported.

3.8. Allergenicity

The manufactured 2'-FL contains no detectable proteins or peptides (see section 3.1) and so would not be anticipated to elicit any allergenic effect.

The Panel considers that the likelihood of adverse allergic reactions to the NFI is low.

4. Discussion

The NFI is 2'-FL, a synthetic trisaccharide consisting of L-fucose, D-galactose and D-glucose, which is produced by using L-fucose and D-lactose as starting raw materials. The NFI is intended to be used in IF, FOF, foods for special medical purposes for infants and young children, and other foods for infants and young children. The NFI is also intended to be used in foods or food supplements for adults.

Considering that 2'-FL is a naturally occurring trisaccharide that is present in human milk, the history of human exposure to 2'-FL is limited primarily to that of BF infants. The Panel notes that the proposed maximum intake level of the NFI by infants is within the range of 2'-FL levels found in mature breast milk, as reported in the literature, although at the high end of this range.

A large proportion of ingested HMO, including 2'-FL, reaches the large intestine where it is either fermented by the intestinal microbiota or excreted unchanged in the faeces. There are no indications that the absorption of the NFI may differ from that of 2'-FL in breast milk and the consumption levels of the NFI would likely be similar to those of 2'-FL in breast milk for infants. Therefore, the Panel considers that the limited amount of the NFI that could be absorbed, as suggested by the data provided, does not raise safety concerns.

Three studies were provided on the potential mutagenicity of the NFI: a bacterial reverse mutation assay (Ames test), an *in vitro* mammalian cell gene mutation test and an *in vitro* micronucleus assay. The Panel considers that the information provided does not raise safety concerns as regards the genotoxicity of the NFI.

Based on observations from a sub-chronic 90-day toxicity study in rats, the Panel considers that the NOAEL is 2 000 mg/kg body weight per day.

The applicant provided an unpublished study report on a double-blind, randomised, controlled clinical trial on the effects of a combination of two oligosaccharides, 2'-FL and LNnT, which are both under safety assessment as NFIs, in infants. This study on the combination of these two oligosaccharides reported that the weight gain of infants who consumed the formula with added 2'-FL and LNnT was not inferior to the weight gain of infants who were fed the control formula up to four months of age. Mean weight, length, head circumference and BMI in infants aged 0-4 months were comparable to the corresponding WHO standard growth curves. The results on stool endpoints and the reported changes in the composition of microbiota in infants consuming the combination of the two oligosaccharides do not raise safety concerns for the studied combination. The Panel considers that this study can be used to draw conclusions on the safety of this combination at the concentrations tested (i.e. 1.0–1.2 g/L of 2'-FL and 0.5–0.6 g/L of LNnT for reconstituted formula). The Panel notes that the concentration of 2'-FL in the infant formula studied in this trial, was only about half of the maximum level as proposed by the applicant. Based on EFSA food consumption data for infants, such a concentration of 2'-FL, would result in about 1.27 g per day and about 209 mg/kg body weight per day, respectively, at the 95th percentile for a 3-months old infant weighing 6 kg.

Taking into account that human milk contains a large number of oligosaccharides, which all together may have an important role for the development of microbiota in infants, the Panel considers that it cannot be excluded that the addition of a single oligosaccharide, i.e. 2'-FL alone, to IF (i.e. without LNnT) may have different effects on infants' growth, microbiota, and stool frequency and consistency from those observed with the studied combination of the two oligosaccharides added to IF in the

provided study. The Panel therefore considers that no conclusion can be drawn from this infant study on the safety of the use of 2′-FL (without LNnT) added to IF.

Noting that complementary feeding of infants and young children results in an increasingly diversified diet, the Panel considers that the potential impact of the addition of 2′-FL alone (without LNnT) or in combination with LNnT on the development of the microbiota diminishes with the proportion of complementary feeding and with age. According to the applicant's intake estimate based on UK data, the 2′-FL intake at the 95th percentile for toddlers corresponds to about 3.4 g per day. On a mg per kg body weight per day basis, the respective value was 247. The Panel considers that 2′-FL alone or in combination with LNnT added at the proposed concentration in foods intended for children older than one year of age, does not raise safety concerns. The Panel considers that the concentration of 2′-FL as applied in the study in infants, should be used in IF, FOF and young-child formula (as described by EFSA (EFSA NDA Panel, 2014)).

The applicant also provided an unpublished study report on a placebo-controlled, double-blind, parallel, dose-response trial in healthy adults who were randomised to consume either 2′-FL or LNnT, alone or in different combinations, or placebo for two weeks. This study showed that participants who consumed, daily, 20 g of 2′-FL reported a significant increase in nausea, rumbling, bloating, passing gas, diarrhoea, loose stools and urgency compared with the placebo group, while for subjects consuming 10 g of 2′-FL per day, these effects were not observed. Considering the highest estimated 95th percentile daily intake of 2′-FL at 5 g for women of child-bearing age plus the maximum intended daily intake from food supplements of 3 g, daily 2′-FL intakes in this population group could theoretically result up to 8 g per day. The Panel notes that the intake estimate is based on the conservative assumption that all proposed food items consumed by an individual actually contain the NFI at the maximum specified level of use.

5. Conclusions

The Panel concludes that the novel food ingredient, 2'-*O*-fucosyllactose (2′-FL):

- is safe for infants (up to one year of age) when added to infant and follow-on formulae, in combination with lacto-*N*-neotetraose (LNnT), at concentrations up to 1.2 g/L of 2′-FL and up to 0.6 g/L of LNnT, at a ratio of 2:1 in the reconstituted formulae;
- is safe for young children (older than one year of age) when added to follow-on and young-child formulae, at concentrations up to 1.2 g/L of 2′-FL (alone or in combination with LNnT, at concentrations up to 0.6 g/L, at a ratio of 2:1);
- is safe when added to other foods at the proposed uses and use levels.

Documentation provided to EFSA

1. Letter from the European Commission to the European Food Safety Authority with the request for an opinion on 2'-*O*-fucosyllactose (2'-FL) as a novel food ingredient. Ref. SANCO/E6/SH/ks D (2015) 37263 Ref. Ares(2015)133857, dated 13 January 2015.

2. Dossier 'Application for the Approval of the Human-identical Milk Oligosaccharide 2'-*O*-fucosyllactose (2'-FL) as a Novel Ingredient for Use in Infant and Follow-on Formulae and in Foods', which was received by EFSA on 19 January 2015. Submitted by Glycom A/S.

3. Initial assessment report carried out by the Food Safety Authority of Ireland: 'Safety assessment of 2'-*O*-fucosyllactose (2'-FL)'.

4. Member States' comments and objections.

5. Response by the applicant to the initial assessment report and the Member States' comments and objections.

6. Additional data were provided by the applicant on 5 and 25 March 2015, 21 and 28 April 2015, 18 May 2015, and 1, 5 and 12 June 2015.

References

Albrecht S, Lane JA, Marino K, Al Busadah KA, Carrington SD, Hickey RM and Rudd PM, 2014. A comparative study of free oligosaccharides in the milk of domestic animals. British Journal of Nutrition, 111, 1313-1328.

Aldredge DL, Geronimo MR, Hua S, Nwosu CC, Lebrilla CB and Barile D, 2013. Annotation and structural elucidation of bovine milk oligosaccharides and determination of novel fucosylated structures. Glycobiology, 23, 664-676.

Asakuma S, Urashima T, Akahori M, Obayashi H, Nakamura T, Kimura K, Watanabe Y, Arai I and Sanai Y, 2008. Variation of major neutral oligosaccharides levels in human colostrum. European Journal of Clinical Nutrition, 62, 488-494.

Asakuma S, Hatakeyama E, Urashima T, Yoshida E, Katayama T, Yamamoto K, Kumagai H, Ashida H, Hirose J and Kitaoka M, 2011. Physiology of consumption of human milk oligosaccharides by infant gut-associated bifidobacteria. Journal of Biological Chemistry, 286, 34583-34592.

Bali V, Panesar PS, Bera MB and Panesar R, 2015. Fructo-oligosaccharides: production, purification and potential applications. Critical Reviews in Food Science and Nutrition, 55, 1475-1490.

Bao Y, Chen C and Newburg DS, 2013. Quantification of neutral human milk oligosaccharides by graphitic carbon high-performance liquid chromatography with tandem mass spectrometry. Analytical Biochemistry, 433, 28-35.

Barrow P, 2007. Toxicology Testing for Products Intended for Pediatric Populations. In: Nonclinical drug safety assessment: practical considerations for successful registration. Eds Sietsema WK and Schwen R. FDA News, Washington, DC, USA, 411-440.

Bode L, 2012. Human milk oligosaccharides: every baby needs a sugar mama. Glycobiology, 22, 1147-1162.

Brand-Miller JC, McVeagh P, McNeil Y and Gillard B, 1995. Human milk oligosaccharides are not digested and absorbed in the small intestine of young infants. Proceedings of the Nutrition Society of Australia, 19, 44.

Brand-Miller JC, McVeagh P, McNeil Y and Messer M, 1998. Digestion of human milk oligosaccharides by healthy infants evaluated by the lactulose hydrogen breath test. Journal of Pediatrics, 133, 95-98.

Castanys-Munoz E, Martin MJ and Prieto PA, 2013. 2'-Fucosyllactose: an abundant, genetically determined soluble glycan present in human milk. Nutrition Reviews, 71, 773-789.

Chaturvedi P, Warren CD, Ruiz-Palacios GM, Pickering LK and Newburg DS, 1997. Milk oligosaccharide profiles by reversed-phase HPLC of their perbenzoylated derivatives. Analytical Biochemistry, 251, 89-97.

Chaturvedi P, Warren CD, Altaye M, Morrow AL, Ruiz-Palacios G, Pickering LK and Newburg DS, 2001a. Fucosylated human milk oligosaccharides vary between individuals and over the course of lactation. Glycobiology, 11, 365-372.

Chaturvedi P, Warren CD, Buescher CR, Pickering LK and Newburg DS, 2001b. Survival of human milk oligosaccharides in the intestine of infants. Advances in Experimental Medicine and Biology, 501, 315-323.

Coppa GV, Pierani P, Zampini L, Carloni I, Carlucci A and Gabrielli O, 1999. Oligosaccharides in human milk during different phases of lactation. Acta Paediatrica, supplement, 88, 89–94.

Coppa GV, Pierani P, Zampini L, Bruni S, Carloni I and Gabrielli O, 2001. Characterization of oligosaccharides in milk and faeces of breast-fed infants by high-performance anion-exchange chromatography. Advances in Experimental Medicine and Biology, 501, 307–314.

Coppa GV, Gabrielli O, Zampini L, Galeazzi T, Ficcadenti A, Padella L, Santoro L, Soldi S, Carlucci A, Bertino E and Morelli L, 2011. Oligosaccharides in 4 different milk groups, Bifidobacteria, and Ruminococcus obeum. Journal of Pediatric Gastroenterology and Nutrition, 53, 80-87.

Coulet M, Phothirath P, Allais L and Schilter B, 2014. Pre-clinical safety evaluation of the synthetic human milk, nature-identical, oligosaccharide 2'-*O*-fucosyllactose (2'FL). Regulatory Toxicology and Pharmacology, 68, 59-69.

Coussement P and Franck A, 2001. Inulin and oligofructose. In: Handbook of dietary fibre. Food science and technology, volume 113). Eds Cho SS and Dreher ML. New York (NY):Marcel Dekker/Boca Raton (FL): CRC Press, 721–736.

Davies PS, Wells JC and Lucas A, 1994. Adjusting milk intake for body size in early infancy. Early Human Development, 36, 61-67.

Dotz V, Rudloff S, Blank D, Lochnit G, Geyer R and Kunz C, 2014. ^{13}C-labeled oligosaccharides in breastfed infants' urine: individual-, structure- and time-dependent differences in the excretion. Glycobiology, 24, 185-194.

EFSA (European Food Safety Authority), 2012. Food Additives Intake Model (FAIM) Template - Version 1.0 - December 2012. EFSA - FIP Unit - FAIM - Instructions for use. http://www.efsa.europa.eu/en/search.htm?search=FAIM&text=FAIM

EFSA AFC Panel (EFSA Panel on Food additives, flavourings, processing aids and materials in contact with food), 2006. Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) related to 2,2-bis(4-hydroxyphenyl)propane. The EFSA Journal, 428, 1-75.

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2014. Scientific Opinion on the essential composition of infant and follow-on formulae. EFSA Journal 2014;12(7):3760, 106 pp. doi:10.2903/j.efsa.2014.3760

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2015. Scientific opinion on the safety of lacto-*N*-neotetraose as a novel food ingredient pursuant to Regulation (EC) No 258/97. EFSA Journal 2015;13(7):4183, 32 pp. doi:10.2903/j.efsa.2015.4183

EMA (European Medicines Agency), 2014. Determination of health based exposure limits. Calculation of a Permitted Daily Exposure (PDE). In: Guideline on setting health based exposure limits for use in risk identification in the manufacture of different medicinal products in shared facilities. (EMA/CHMP/ CVMP/ SWP/169430/2012). Committee for Medicinal Products for Human Use (CHMP), Committee for Medicinal Products for Veterinary Use (CVMP), p. 4. Available online: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2014/11/WC500177735.pdf

Engfer MB, Stahl B, Finke B, Sawatzki G and Daniel H, 2000. Human milk oligosaccharides are resistant to enzymatic hydrolysis in the upper gastrointestinal tract. American Journal of Clinical Nutrition, 71, 1589-1596.

Erney RM, Malone WT, Skelding MB, Marcon AA, Kleman-Leyer KM, O'Ryan ML, Ruiz-Palacios G, Hilty MD, Pickering LK and Prieto PA, 2000. Variability of human milk neutral oligosaccharides in a diverse population. Journal of Pediatric Gastroenterology and Nutrition, 30, 181-192.

Erney R, Hilty M, Pickering L, Ruiz-Palacios G and Prieto P, 2001. Human milk oligosaccharides: a novel method provides insight into human genetics. Advances in Experimental Medicine and Biology, 501, 285-297.

Gabrielli O, Zampini L, Galeazzi T, Padella L, Santoro L, Peila C, Giuliani F, Bertino E, Fabris C and Coppa GV, 2011. Preterm milk oligosaccharides during the first month of lactation. Pediatrics, 128, e1520-1531.

Galeotti F, Coppa GV, Zampini L, Maccari F, Galeazzi T, Padella L, Santoro L, Gabrielli O and Volpi N, 2012. On-line high-performance liquid chromatography-fluorescence detection-electrospray ionization-mass spectrometry profiling of human milk oligosaccharides derivatized with 2-aminoacridone. Analytical Biochemistry, 430, 97-104.

Galeotti F, Coppa GV, Zampini L, Maccari F, Galeazzi T, Padella L, Santoro L, Gabrielli O and Volpi N, 2014. Capillary electrophoresis separation of human milk neutral and acidic oligosaccharides derivatized with 2-aminoacridone. Electrophoresis, 35, 811-818.

Gnoth MJ, Kunz C, Kinne-Saffran E and Rudloff S, 2000. Human milk oligosaccharides are minimally digested in vitro. Journal of Nutrition, 130, 3014-3020.

Gnoth MJ, Rudloff S, Kunz C and Kinne RK, 2001. Investigations of the in vitro transport of human milk oligosaccharides by a Caco-2 monolayer using a novel high performance liquid chromatography-mass spectrometry technique. Journal of Biological Chemistry, 276, 34363-34370.

Havenaar R and Van Dokkum W, 2001. Lactulose: a review on effects, clinical results, and safety aspects in relation to its influence on the colonic environment. Food Science and Technology, 113, 179-194.

Heaney RP, 2013. Dairy intake, dietary adequacy, and lactose intolerance. Advances in Nutrition, 4, 151-156.

Hester SN, Hustead DS, Mackey AD, Singhal A and Marriage BJ, 2012. Is the macronutrient intake of formula-fed infants greater than breast-fed infants in early infancy? Journal of Nutrition and Metabolism, 2012, 891201.

Hong Q, Ruhaak LR, Totten SM, Smilowitz JT, German JB and Lebrilla CB, 2014. Label-free absolute quantitation of oligosaccharides using multiple reaction monitoring. Analytical Chemistry, 86, 2640-2647.

ICH (International Conference on Harmonisation), 2011. ICH harmonised tripartite guideline. Impurities: guideline for residual solvents: Q3C(R5). Current step 4 version, dated 4 February 2011, parent guideline dated 17 July 1997. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, Geneva, Switzerland. Available online: www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q3C/Step4/Q3C_R5_Step4.pdf

Ishizuka Y, Nemoto T, Fujiwara M, Fujita KI and Nakanishi H, 1999. Three-dimensional structure of fucosyllactoses in an aqueous solution. Journal of Carbohydrate Chemistry, 18, 523-533.

Kunz C and Rudloff S, 1993. Biological functions of oligosaccharides in human milk. Acta Paediatrica, 82, 903-912.

Leo F, Asakuma S, Fukuda K, Senda A and Urashima T, 2010. Determination of sialyl and neutral oligosaccharide levels in transition and mature milks of Samoan women, using anthranilic derivatization followed by reverse phase high performance liquid chromatography. Bioscience, Biotechnology, and Biochemistry, 74, 298-303.

Mattar R, de Campos Mazo DF and Carrilho FJ, 2012. Lactose intolerance: diagnosis, genetic, and clinical factors. Clinical and Experimental Gastroenterology, 5, 113-121.

Meyrand M, Dallas DC, Caillat H, Bouvier F, Martin P and Barile D, 2013. Comparison of milk oligosaccharides between goats with and without the genetic ability to synthesize alpha-casein. Small Ruminant Research, 113, 411-420.

Morrow AL, Ruiz-Palacios GM, Altaye M, Jiang X, Guerrero ML, Meinzen-Derr JK, Farkas T, Chaturvedi P, Pickering LK and Newburg DS, 2004. Human milk oligosaccharides are associated with protection against diarrhea in breast-fed infants. Journal of Pediatrics, 145, 297-303.

Musumeci M, Simpore J, D'Agata A, Sotgiu S and Musumeci S, 2006. Oligosaccharides in colostrum of Italian and Burkinabe women. Journal of Pediatric Gastroenterology and Nutrition, 43, 372-378.

Nakhla T, Fu D, Zopf D, Brodsky NL and Hurt H, 1999. Neutral oligosaccharide content of preterm human milk. British Journal of Nutrition, 82, 361-367.

NatCen Social Research, Medical Research Council, Resource Centre for Human Nutrition Research, Medical Research Council, Epidemiology and Medical Care Unit, University of Newcastle upon Tyne, Institute for Ageing and Health, and Human Nutrition Research Centre, 2013. Diet and Nutrition Survey of Infants and Young Children (DNSIYC) 2011 [computer file]. (SN 7263), Prepared by London, Engl., National Centre for Social Research (NatCen) / Cambridge, UK, Medical Research Council (MRC), Human Nutrition Research / Cambridge, UK, MRC Epidemiology Unit / Cambridge, UK, MRC Epidemiology Unit / Human Nutrition Research Centre (HNRC), Newcastle University for Department of Health / London, UK, Food Standards Agency (FSA) and Distributed by Colchester, UK, UK Data Archive. Available online: http://esds.ac.uk/doi/?sn=7263 [Last modified date: 16 May 2013; open full catalogue record].

Newburg DS, 2013. Glycobiology of human milk. Biochemistry, 78, 771-785.

Obermeier S, Rudloff S, Pohlentz G, Lentze MJ and Kunz C, 1999. Secretion of ^{13}C-labelled oligosaccharides into human milk and infant's urine after an oral ^{13}C-galactose load. Isotopes in Environmental and Health Studies, 35, 119-125.

OECD (Organisation for Economic Co-operation and Development), 1997a. Test No. 471: Bacterial reverse mutation test. In: OECD guidelines for the testing of chemicals, Section 4: Health effects, 11 pp.

OECD (Organisation for Economic Co-operation and Development), 1997b. Test No. 476: *In vitro* mammalian cell gene mutation test. In: OECD guidelines for the testing of chemicals, Section 4: Health effects, 10 pp.

OECD (Organisation for Economic Co-operation and Development), 1998a. Test No. 408: Repeated dose 90-day oral toxicity study in rodents. In: OECD guidelines for the testing of chemicals, Section 4: Health effects, 10 pp.

OECD, (Organisation for Economic Co-operation and Development), 1998b. OECD Principles of good laboratory practice (as revised in 1997). OECD series on principles of good laboratory practice and compliance monitoring, number 1, ENV/MC/CHEM(98)17, 41 pp.

OECD (Organisation for Economic Co-operation and Development), 2014. Test No. 487: *In vitro* mammalian cell micronucleus test. In: OECD guidelines for the testing of chemicals, Section 4: Health effects, 23 pp.

Rudloff S and Kunz C, 2012. Milk oligosaccharides and metabolism in infants. Advances in Nutrition, 3, 398S-405S.

Rudloff S, Obermeier S, Borsch C, Pohlentz G, Hartmann R, Brosicke H, Lentze MJ and Kunz C, 2006. Incorporation of orally applied ^{13}C-galactose into milk lactose and oligosaccharides. Glycobiology, 16, 477-487.

Rudloff S, Pohlentz G, Borsch C, Lentze MJ and Kunz C, 2012. Urinary excretion of *in vivo* ^{13}C-labelled milk oligosaccharides in breastfed infants. British Journal of Nutrition, 107, 957-963.

Smilowitz JT, O'Sullivan A, Barile D, German JB, Lonnerdal B and Slupsky CM, 2013. The human milk metabolome reveals diverse oligosaccharide profiles. Journal of Nutrition, 143, 1709-1718.

Sumiyoshi W, Urashima T, Nakamura T, Arai I, Saito T, Tsumura N, Wang B, Brand-Miller J, Watanabe Y and Kimura K, 2003. Determination of each neutral oligosaccharide in the milk of Japanese women during the course of lactation. British Journal of Nutrition, 89, 61-69.

Tao N, DePeters EJ, German JB, Grimm R and Lebrilla CB, 2009. Variations in bovine milk oligosaccharides during early and middle lactation stages analyzed by high-performance liquid chromatography-chip/mass spectrometry. Journal of Dairy Science, 92, 2991-3001.

Tao N, Ochonicky KL, German JB, Donovan SM and Lebrilla CB, 2010. Structural determination and daily variations of porcine milk oligosaccharides. Journal of Agricultural and Food Chemistry, 58, 4653-4659.

Thurl S, Henker J, Siegel M, Tovar K and Sawatzki G, 1997. Detection of four human milk groups with respect to Lewis blood group dependent oligosaccharides. Glycoconjugate Journal, 14, 795-799.

Thurl S, Munzert M, Henker J, Boehm G, Muller-Werner B, Jelinek J and Stahl B, 2010. Variation of human milk oligosaccharides in relation to milk groups and lactational periods. British Journal of Nutrition, 104, 1261-1271.

Torres DPM, Gonçalves MDPF, Teixeira JA and Rodrigues LR, 2010. Galactooligosaccharides: production, properties, applications, and significance as prebiotics. Comprehensive Reviews in Food Science and Food Safety, 9, 438-454.

UKDA (UK Data Archive), 2012. National Diet and Nutrition Survey, 2008–2010 [computer file], 3rd edition. (SN: 6533), Colchester, Essex, UK Data Archive (UK) [distributor]. Available online: http://dx.doi.org/10.5255/UKDA-SN-6533-2

Unpublished study report, 2015a. Dated 27 February 2015. Wil Research Europe B.V., s'Hertogenbosch, The Netherlands. Study Title: 'An *in vitro* micronucleus assay with 2'-*O*-fucosyllactose in cultured peripheral human lymphocytes' (Project 507397).

Unpublished study report, 2015b. Dated: 25 February 2015. Study Title: 'Clinical safety of a new infant starter formula containing 2 human milk oligosaccharides (HMOs), Part 1' (Study number 11.26.INF).

Unpublished study report, 2015c. Dated: 1 June 2015. Study Title: 'The effects of human-milk-oligosaccharides on the faecal microbiota and on gastrointestinal symptoms in healthy adult volunteers' (Study number 2014-1).

Unpublished study report, 2015d. Dated: 3 June 2015. Study title: 'Stool microbiota and metabolic signature in infants at 3 months of age who were fed a starter infant formula supplemented with 2'FL and LNnT' (Study number 11.26INF).

Urashima T, Bubb WA, Messer M, Tsuji Y and Taneda Y, 1994. Studies of the neutral trisaccharides of goat (Capra hircus) colostrum and of the one- and two-dimensional 1H and 13C NMR spectra of 6'-N-acetylglucosaminyllactose. Carbohydrate Research, 262, 173-184.

WHO (World Health Organisation), 2014. WHO Child Growth Standards. Geneva, Switz., WHO Multicentre Growth Reference Study Group, World Health Organization (WHO). Available online: http://www.who.int/childgrowth/standards/weight_for_age/en/index.html

Abbreviations

2′-FL	2′-*O*-fucosyllactose
BF	breast-fed
CI	confidence interval
DNSIYC	diet and nutrition survey on infants and young children
EMA	European Medicines Agency
FAIM	food additive intakes model
FOF	follow-on formula(e)
GLP	good laboratory practices
HACCP	hazards analysis and critical control points
HMO	human milk oligosaccharide
HPLC	high-performance liquid chromatography
IF	infant formula(e)
ITT	intention-to-treat
LNnT	lacto-*N*-neotetraose
NDNS	national diet and nutrition survey
NFI	novel food ingredient
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
OR	odds ratio
PARNUTS	foodstuffs for particular nutritional uses
PND	post-natal day
PP	per-protocol
UHT	ultra-high temperature
WHO	World Health Organization