SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 29 April 2022

doi: 10.2903/j.efsa.2022.7329

Safety of 3-fucosyllactose (3-FL) produced by a derivative strain of *Escherichia coli* BL21 (DE3) as a Novel Food pursuant to Regulation (EU) 2015/2283

EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA),
Dominique Turck, Torsten Bohn, Jacqueline Castenmiller, Stefaan De Henauw,
Karen Ildico Hirsch-Ernst, Alexandre Maciuk, Inge Mangelsdorf, Harry J McArdle,
Androniki Naska, Carmen Pelaez, Kristina Pentieva, Alfonso Siani, Frank Thies,
Sophia Tsabouri, Marco Vinceti, Francesco Cubadda, Thomas Frenzel, Marina Heinonen,
Rosangela Marchelli, Monika Neuhäuser-Berthold, Morten Poulsen, Miguel Prieto Maradona,
Josef Rudolf Schlatter, Henk van Loveren, Paolo Colombo, Estefanía Noriega Fernández and
Helle Katrine Knutsen

Abstract

Following a request from the European Commission, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver an opinion on 3-fucosyllactose (3-FL) as a novel food (NF) pursuant to Regulation (EU) 2015/2283. The NF is mainly composed of the human-identical milk oligosaccharide (HiMO) 3-FL, but it also contains D-lactose, L-fucose, D-glucose and D-galactose, and a small fraction of other related saccharides. The NF is produced by fermentation with a genetically modified strain of *Escherichia coli* BL21 (DE3). The information provided on the manufacturing process, composition and specifications of the NF does not raise safety concerns. The applicant intends to add the NF to a variety of foods, including infant formula and follow-on formula, food for infants and young children, food for special medical purposes and food supplements. The target population is the general population. The anticipated daily intake of 3-FL from both proposed and combined (authorised and proposed) uses at their respective maximum use levels in all population categories does not exceed the highest intake level of 3-FL from human milk in infants on a body weight basis. The intake of 3-FL in breastfed infants on a body weight basis is expected to be safe also for other population groups. The intake of other carbohydrate-type compounds structurally related to 3-FL is also considered of no safety concern. Food supplements are not intended to be used if other foods with added 3-FL or human milk are consumed on the same day. The Panel concludes that the NF is safe under the proposed conditions of use.

Keywords: 3-fucosyllactose, 3-FL, human milk oligosaccharide, HMO, HiMO, novel food, safety

Requestor: European Commission

Question number: EFSA-Q-2020-00309

Correspondence: nif@efsa.europa.eu

Panel members: Dominique Turck, Torsten Bohn, Jacqueline Castenmiller, Stefaan De Henauw, Karen Ildico Hirsch-Ernst, Helle Katrine Knutsen, Alexandre Maciuk, Inge Mangelsdorf, Harry J McArdle, Androniki Naska, Carmen Pelaez, Kristina Pentieva, Alfonso Siani, Frank Thies, Sophia Tsabouri and Marco Vinceti.

Declarations of interest: The declarations of interest of all scientific experts active in EFSA's work are available at https://ess.efsa.europa.eu/doi/doiweb/doisearch.

Acknowledgments: The Panel wishes to thank EFSA Staff Reinhard Ackerl, Petra Gergelova and Emanuela Turla for the support provided to this scientific output.

Suggested citation: EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens), Turck D, Bohn T, Castenmiller J, De Henauw S, Hirsch-Ernst KI, Maciuk A, Mangelsdorf I, McArdle HJ, Naska A, Pelaez C, Pentieva K, Siani A, Thies F, Tsabouri S, Vinceti M, Cubadda F, Frenzel T, Heinonen M, Marchelli R, Neuhäuser-Berthold M, Poulsen M, Prieto Maradona M, Schlatter JR, van Loveren H, Colombo P, Noriega Fernández E and Knutsen HK, 2022. Scientific Opinion on the safety of 3-fucosyllactose (3-FL) produced by a derivative strain of *Escherichia coli* BL21 (DE3) as a Novel Food pursuant to Regulation (EU) 2015/2283. EFSA Journal 2022;20(5):7329, 23 pp. https://doi.org/10.2903/j.efsa.2022.7329

ISSN: 1831-4732

Table of contents

1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

On 17 March 2020, the company Chr. Hansen A/S submitted a request to the Commission in accordance with Article 10 of Regulation (EU) 2015/2283[1] to place 3-fucosyllactose (3-FL) on the EU market.

3-FL is intended to be used in a number of food categories including infant formula (IF) and follow-on formula (FOF), processed cereal-based food and baby food for infants and young children, food for infants and young children for special medical purposes, and food for special medical purposes (FSMP) as defined in Regulation (EU) No 609/2013[2], milk-based drinks and similar products intended for young children, and food supplements (FS) as defined in Directive 2002/46/EC[3].

The applicant has requested data protection according to the provisions of Article 26 of Regulation (EU) 2015/2283.

In accordance with Article 10(3) of Regulation (EU) 2015/2283, the European Commission asks the European Food Safety Authority to provide a scientific opinion on 3-FL.

1.2. Additional information

3-FL is included in the Union list of authorised novel foods (NFs) (Commission Implementing Regulation (EU) 2017/2470 of 20 December 2017[4]) when produced by fermentation with a genetically modified strain of *Escherichia coli* K-12 MG1655. Since 2015, several scientific opinions have been adopted by the EFSA NDA Panel on the safety of human-identical milk oligosaccharides (HiMOs) as NFs pursuant to Regulation (EC) No 258/97 or Regulation (EU) 2015/2283: 2′-fucosyllactose (2′-FL) (EFSA NDA Panel, 2015a), lacto-N-neotetraose (LNnT) (EFSA NDA Panel, 2015b), LNnT and 2′-FL in FS for children (EFSA NDA Panel, 2015c), N-acetyl-D-neuraminic acid (NANA) (EFSA NDA Panel, 2017), 2′-FL/difucosyllactose (DFL) mixture (EFSA NDA Panel, 2019a), lacto-N-tetraose (LNT) produced with a derivative strain of *E. coli* K-12 DH1 (EFSA NDA Panel, 2019b), 3′-sialyllactose (3′-SL) sodium salt (EFSA NDA Panel, 2020a), 6′-sialyllactose (6′-SL) sodium salt (EFSA NDA Panel, 2020b), LNnT produced with derivative strains of *E. coli* BL21 (DE3) (EFSA NDA Panel, 2020c), 3-FL produced with a derivative strain of *E. coli* K-12 MG1655 (EFSA NDA Panel, 2021), 2′-FL/DFL mixture and LNT in FS for infants (EFSA NDA Panel, 2022a), 2′-FL and LNnT in FS for infants (EFSA NDA Panel, 2022b) and LNT produced with derivative strains of *E. coli* BL21 (DE3) (EFSA NDA Panel, 2022c).

2. Data and methodologies

2.1. Data

The safety assessment of this NF is based on data supplied in the application and information submitted by the applicant following an EFSA request for supplementary information.

During the assessment, the Panel identified additional data which were not included in the application.

Administrative and scientific requirements for NF applications referred to in Article 10 of Regulation (EU) 2015/2283 are listed in the Commission Implementing Regulation (EU) 2017/2469[5].

[1] Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001. OJ L 327, 11.12.2015, p. 1–22.

[2] Regulation (EU) No 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control and repealing Council Directive 92/52/EEC, Commission Directives 96/8/EC, 1999/21/EC, 2006/125/EC and 2006/141/EC, Directive 2009/39/EC of the European Parliament and of the Council and Commission Regulations (EC) No 41/2009 and (EC) No 953/2009. OJ L 181, 29.6.2013, p. 35–56.

[3] 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 food supplements. OJ L 183, 12.7.2002, p. 51–57.

[4] Commission Implementing Regulation (EU) 2017/2470 of 20 December 2017 establishing the Union list of novel foods in accordance with Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods. OJ L 351, 30.12.2017, p. 72–201.

[5] Commission Implementing Regulation (EU) 2017/2469 of 20 December 2017 laying down administrative and scientific requirements for applications referred to in Article 10 of Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods. OJ L 351, 30.12.2017, p. 64–71.

A common and structured format on the presentation of NF applications is described in the EFSA guidance on the preparation and presentation of a NF application (EFSA NDA Panel, 2016). As indicated in this guidance, it is the duty of the applicant to provide all of the available (proprietary, confidential and published) scientific data (both in favour and not in favour) that are pertinent to the safety of the NF.

This NF application includes a request for protection of proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283. The data requested by the applicant to be protected comprise: (i) identity of the NF; (ii) toxicological information; (iii) information on the genetically modified production strain; (iv) method validation reports for the determination of the carbohydrate content in the NF.

2.2. Methodologies

The assessment follows the methodology set out in the EFSA guidance on NF applications (EFSA NDA Panel, 2016) and the principles described in the relevant existing guidance documents from the EFSA Scientific Committee. The legal provisions for the assessment are laid down in Article 11 of Regulation (EU) 2015/2283 and in Article 7 of the Commission Implementing Regulation (EU) 2017/2469. The legal provisions for the assessment of food intended for infants and young children and FSMP are laid down in Regulation (EU) No 609/2013 and, respectively, in Commission Delegated Regulation (EU) 2016/128[6] (FSMP), and in Commission Delegated Regulation (EU) 2016/127[7] (as regards the specific compositional and information requirements for IF and FOF and as regards requirements on information relating to infant and young child feeding).

This assessment concerns only the risks that might be associated with consumption of the NF under the proposed conditions of use and is not an assessment of the efficacy of the NF with regard to any claimed benefit. Furthermore, this assessment also is not an assessment on whether the NF is suitable as stipulated by Regulation (EU) No 609/2013.

3. Assessment

3.1. Introduction

The NF, which is the subject of the application, contains 3-FL as primary constituent (≥ 90% w/w dry matter (DM)), a fucosylated neutral oligosaccharide consisting of L-fucose linked via an α-(1-3) bond to the D-glucose moiety of D-lactose. 3-FL has been identified as a relevant component of the complex fraction of oligosaccharides naturally occurring in mammalian milk, with the highest levels present in human milk, and therefore is typically acknowledged as a human milk oligosaccharide (HMO). The Panel notes that although 3-FL is the major component of the NF, it also contains D-lactose, L-fucose, D-glucose and D-galactose, and a small fraction of other related saccharides. The NF is produced by fermentation with a derivative strain of *E. coli* BL21 (DE3).

The NF is proposed to be used in food for infants and young children (including IF, FOF, processed cereal-based food and baby food as defined in Regulation (EU) No 609/2013), FSMP as defined in Regulation (EU) No 609/2013 and FS as defined in Directive 2002/46/EC. The target population is the general population.

3-FL produced with a derivative strain of *E. coli* K-12 MG1655 has been previously assessed by EFSA as a NF with a positive outcome (EFSA NDA Panel, 2021). In addition, 2′-FL and LNnT (EFSA NDA Panel, 2020c), produced with derivative strains of the same host strain *E. coli* BL21 (DE3), have been authorised as NFs in the European Union (Commission Implementing Regulation 2017/2470), and LNT produced with derivative strains of *E. coli* BL21 (DE3) has recently been assessed by EFSA with a positive outcome (EFSA NDA Panel, 2022c).

According to Article 3(2)(a) of Regulation (EU) 2015/2283, the NF falls under the following categories:

[6] Commission Delegated Regulation (EU) 2016/128 of 25 September 2015 supplementing Regulation (EU) No 609/2013 of the European Parliament and of the Council as regards the specific compositional and information requirements for food for special medical purposes. OJ L 25, 2.2.2016, p. 30–43.

[7] Commission Delegated Regulation (EU) 2016/127 of 25 September 2015 supplementing Regulation (EU) No 609/2013 of the European Parliament and of the Council as regards the specific compositional and information requirements for infant formula and follow-on formula and as regards requirements on information relating to infant and young child feeding. OJ L 25, 2.2.2016, p. 1–29.

i) 'food with a new or intentionally modified molecular structure, where that structure was not used as, or in, a food within the Union before 15 May 1997'; and
ii) 'food consisting of, isolated from or produced from microorganisms, fungi or algae'.

3.2. Identity of the NF

The NF is a powdered mixture mainly composed of 3-FL (≥ 90.0% w/w DM), which is one of the most abundant neutral core HMOs (Erney et al., 2001; Thurl et al., 2017). The NF also contains D-lactose (≤ 5% w/w DM), L-fucose (≤ 3% w/w DM), D-galactose (≤ 3% w/w DM) and D-glucose (≤ 3% w/w DM) and a small fraction of other related saccharides (sum of other carbohydrates ≤ 5% w/w DM). It is produced by fermentation with a genetically modified strain of *E. coli* BL21 (DE3). 3-FL is a trisaccharide consisting of L-fucose linked via an α-(1-3) bond to the D-glucose moiety of D-lactose (Table 1 and Figure 1). 3-FL is a constitutional isomer of 2′-FL, which contains the same monosaccharide moieties as those present in 3-FL but with an α-(1-2′) bond between L-fucose and the D-galactose moiety of D-lactose.

Table 1: Chemical identity of 3-FL

Chemical substance	
Chemical (IUPAC) name	β-D-Galactopyranosyl-(1→4)-[-α-L-fucopyranosyl-(1→3)]-D-glucopyranose
Common name	3-Fucosyllactose
Abbreviations	3-FL, 3FL, 3F, 3fl, 3-fl, JW-3FL
Alternative chemical names	• β-D-Gal-(1-4)-[α-L-Fuc-(1-3)]-D-Glc • α-L-Fuc-(1→3)-[β-D-Gal-(1→4)]-D-Glc • α-L-Fucosypyranosyl-(1→3)-[β-D-galactopyranosyl-(1→4)]-D-glucopyranoside
CAS Number	41312-47-4
Molecular formula	$C_{18}H_{32}O_{15}$
Molecular weight	488.44 Da

The molecular structure of 3-FL has been determined by high-performance liquid chromatography – electrospray ionisation – tandem mass spectrometry (HPLC-ESI-MS/MS), based on its collision-induced decay (CID) fragmentation pattern and multiple reaction monitoring (MRM) analysis, by comparison with high purity in-house and commercially available standards, which allowed to differentiate between 3-FL α-(1-3) and 2′-FL α-(1-2′). The mass fragmentation pattern is consistent with that reported in the literature (Zaia, 2004; Kailemia et al., 2014; Wang et al., 2016; Yamagaki and Makino, 2017; Mank et al., 2019).

The identity of 3-FL was also confirmed by high-performance anion-exchange chromatography – pulsed amperometric detection (HPAEC-PAD) by comparison with a high purity in-house standard.

The structure of 3-FL has been confirmed by mono-dimensional (1D) nuclear magnetic resonance (NMR) spectroscopy including ^{1}H-, ^{13}C- and ^{13}C-DEPT-135 (distortionless enhancement by polarisation transfer) spectra, and two-dimensional (2D) NMR spectroscopy, including ^{1}H-^{1}H COSY (correlated spectroscopy), ^{1}H-^{13}C HSQC (heteronuclear single quantum correlation) and ^{1}H-^{13}C HMBC (heteronuclear multiple-bond correlation) spectra, by comparison with a commercially available 3-FL standard. In the HMBC spectrum, the ^{3}J coupling constant of the H-1″ of the L-fucose to the D-glucose C-3 has been recognised and assigned.

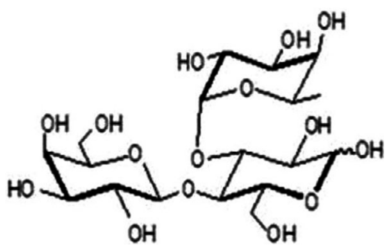

Figure 1: Chemical structure of 3-FL (EFSA NDA Panel, 2021)

The 3-FL produced by the microbial fermentation described has been shown to be chemically and structurally identical to a commercially available 3-FL derived from human milk by 1D and 2D NMR spectroscopy, and the Panel considers it as being a HiMO.

3.3. Production process

According to the information provided by the applicant, the NF is produced in line with Good Manufacturing Practice (GMP) and Hazard Analysis Critical Control Points (HACCP) principles, in a facility that is ISO:9001 and FSSC 22000 certified.

The NF is produced by a two-step fed-batch fermentation process using a genetically modified strain derived from the host strain *E. coli* BL21 (DE3). The production strain *E. coli* BL21 (DE3) JBT-3-FL has been modified to effectively synthesise 3-FL. Glycerol, glucose and/or sucrose can be used as carbon sources for the cultivation of the production strain and lactose is utilised as a substrate for the production of 3-FL. The process is carried out without inhibitors, inducers or antibiotics and no solvents are used except water. The duration of the fermentation step is set to optimise the concentration of 3-FL. At the end of the fermentation process, the bacterial biomass is removed from the final product by centrifugation and ultrafiltration. The isolation, purification and concentration of the product involve several filtration, ion removal and decolourisation steps. All chemicals used in the process are of food-grade quality. Other processing aids, such as ion exchange resins, activated carbon and filtration membranes, are also in conformance with the manufacture of food. The concentrated purified 3-FL is spray-dried to obtain a powder form.

The production strain *E. coli* BL21 (DE3) JBT-3-FL is a genetically modified derivative of the host strain *E. coli* BL21 (DE3) (F^- *ompT* *hsdS*$_B$ ($r_B^- m_B^-$) *gal dcm* (DE3)). The *E. coli* BL21 (DE3) strain was developed through T7 RNA polymerase-based gene expression by introducing a lambda prophage containing a T7 RNA polymerase under the control of *lac*UVA promoter and it is typically used in laboratories worldwide. *E. coli* BL21 (DE3) is considered to be non-pathogenic and unlikely to survive in host tissues or to cause disease (Chart et al., 2000). The genome sequence of *E. coli* BL21 (DE3) showed the absence of genes encoding invasion factors, adhesion molecules and enterotoxins associated with virulence (Jeong et al., 2009). The production strain has been deposited at the German Collection of Microorganisms and Cell Cultures (DSMZ). A detailed description of the genetic modification steps applied to obtain the production strain has been provided by the applicant. No residual DNA from the production strain was detected in the NF using quantitative polymerase chain reaction (qPCR) amplification of five antimicrobial resistance genes introduced during the genetic modification of the production strain. The absence of both DNA and viable cells from the production strain has been demonstrated in accordance with the EFSA Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018).

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

3.4. Compositional data

Batch-to-batch analyses showed that the NF consists of 3-FL as primary ingredient (96.5% w/w DM[8]). The remainder is a mixture of substances[8,9] such as D-lactose (0.7% w/w DM), L-fucose (0.6% w/w DM), D-galactose (< 0.14% w/w DM) and D-glucose (< 0.14% w/w DM). In addition, the NF contains other carbohydrates individually present at low concentration (sum of other carbohydrates, 1.9% w/w DM[8,9]). D-Lactose is the most abundant molecule in human milk (~ 7 g/100 mL) and its monomers D-glucose and D-galactose are normal constituents of human milk. L-Fucose is also found in human milk (Smilowitz et al., 2013) at concentrations ranging from 20 to 30 mg/L (Choi et al., 2015).

With regard to the physico-chemical properties, the NF can be described as a white- to ivory-coloured spray-dried powder. It is readily soluble in aqueous solutions (min. 500 g/L at ambient temperature).

In order to confirm that the manufacturing process is reproducible and adequate to produce on a commercial scale a product with certain required characteristics, the applicant provided analytical information for five batches of the NF (Table 2). Information was provided on the accreditation of the laboratories that conducted the analyses presented in the application.

[8] Average content in five batches of the NF.
[9] For those batches of the NF where the levels of any carbohydrate by-product were below the respective limit of quantification (LOQ), the concentration of the corresponding compound has been considered to be equal to the respective LOQ value for the purpose of calculating its average content in the five batches of the NF and the sum of other carbohydrates.

Table 2: Batch-to-batch analysis of the NF

Parameter	Batch number					Method of analysis
	#1	#2	#3	#4	#5	
Composition						
3-FL (% w/w DM)	96.0	96.0	98.8	94.1	97.4	HPAEC-PAD (validated internal method)[1]
D-Lactose (% w/w DM)	0.7	0.5	0.6	1.1	0.5	
D-Glucose (% w/w DM)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	
D-Galactose (% w/w DM)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	
L-Fucose (% w/w DM)	0.7	0.5	0.6	< LOQ	0.9	
Sum of other carbohydrates (% w/w DM)	2.0	2.4	0.0	4.2	0.8	Calculation[2]
Protein (%)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	Nanoquant (modified Bradford)
Ash (%)	0.36	0.33	0.25	0.02	0.12	ASU L 06.00-4
Water (%)	7.6	8.0	8.5	7.7	7.5	Karl-Fischer titration
Contaminants						
Arsenic[3] (mg/kg)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	ASU L 00.00-135: 2011-01 – ICP-MS
Cadmium[3] (mg/kg)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	
Lead[3] (mg/kg)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	
Mercury[3] (mg/kg)	< LOQ	< LOQ	< LOQ	0.006	< LOQ	
Aflatoxin M1 (μg/kg)	< 0.025	< 0.025	< 0.025	< 0.025	< 0.025	DIN EN ISO 14501: 2008-01 – IAC-HPLC-FD
Aflatoxin B1 (μg/kg)	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	DIN EN ISO 16050:2011-09 – IAC-HPLC – post column derivatisation
Aflatoxin B2 (μg/kg)	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	
Aflatoxin G1 (μg/kg)	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	
Aflatoxin G2 (μg/kg)	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	
Sum of aflatoxins B1, B2, G1 and G2 (μg/kg)	< 0.40	< 0.40	< 0.40	< 0.40	< 0.40	
Microbial parameters						
Standard Plate Count (CFU/g)	< 10	< 10	< 10	< 10	< 10	ISO 4833-2
Yeast and mould (CFU/g)	< 10	< 10	< 10	< 10	< 10	ISO 21527-2: 2008-07
Enterobacteriaceae (CFU/g)	< 10	< 10	< 10	< 10	< 10	ISO 21528-2: 2019-05
Enterobacteriaceae (in 10 g)	ND	ND	ND	ND	ND	ISO 21528-1: 2017-09
Salmonella (in 25 g)	ND	ND	ND	ND	ND	DIN EN ISO 6579-1: 2017-07
Cronobacter spp. (in 10 g)	ND	ND	ND	ND	ND	ISO/TS 22964: 2017-04
Listeria monocytogenes (in 25 g)	ND	ND	ND	ND	ND	DIN EN ISO 11290-1: 2017-09
Bacillus cereus (CFU/g)	< 50	< 50	< 50	< 50	< 50	DIN EN ISO 7932: 2020-11
Endotoxins (EU/mg)	0.007	0.009	0.023	< 0.005	< 0.005	Ph. Eur. 2.6.14

3-FL: 3-Fucosyllactose; ASU: Official collection of analysis methods according to § 64 of the German Food and Feed Code (LFGB); CFU: Colony forming unit; DIN: German Institute for Standardisation e. V.; DM: Dry matter; EN: European norm; EU: Endotoxin unit; HPAEC-PAD: High-performance anion-exchange chromatography – pulsed amperometric detection; IAC-HPLC-FD: Immunoaffinity chromatography – high-performance liquid chromatography – fluorescence detection; ICP-MS: Inductively coupled plasma – mass spectrometry; ISO: International Organisation for Standardisation; LOQ: Limit of quantification; ND: Not detected; Ph. Eur.: European Pharmacopeia; TS: Technical specification.

(1): LOQs: D-Lactose = 0.14% w/w DM; D-Galactose = 0.14% w/w DM; D-Glucose = 0.14% w/w DM; L-Fucose = 0.28% w/w DM.

(2): Sum of other carbohydrates = 100 (% w/w DM) – 3-FL (% w/w DM) – Quantified carbohydrates (% w/w DM) – Ash (% w/w DM). For those batches of the NF where the levels of any carbohydrate by-product were below the respective limit of quantification (LOQ), the concentration of the corresponding compound has been considered to be equal to the respective LOQ value for the purpose of calculating the sum of other carbohydrates in the corresponding batch.

(3): LOQs: Arsenic = 0.05 mg/kg; Cadmium = 0.010 mg/kg; Lead = 0.010 mg/kg; Mercury = 0.005 mg/kg.

The Panel considers that the information provided on the composition of the NF is sufficient and does not raise safety concerns.

3.4.1. Stability

Stability of the NF

The applicant performed stability tests on one batch of a HiMO mixture containing 2′-FL (47.7% w/w DM), 3-FL (15.1% w/w DM), LNT (24.7% w/w DM), 3′-SL sodium salt (4.3% w/w DM), 6′-SL sodium salt (5.6% w/w DM) and other carbohydrates (5.7% w/w DM). The applicant stated that 3-FL included in the HiMO mixture was manufactured according to the production process described in Section 3.3. The tests were carried out at normal (25°C and 60% relative humidity (RH)) and accelerated (40°C and 75% RH) storage conditions for a period of 104 and 26 weeks, respectively. The samples were analysed for 3-FL (HPAEC-PAD) and moisture (Karl-Fischer titration) content. Upon EFSA's request for additional information, the applicant provided stability data up to 156-week storage under normal conditions, also reporting the concentration of the individual carbohydrates present in the HiMO mixture throughout the storage period.

The content of 3-FL (expressed on a DM basis) remained relatively stable over the 156-week period under normal storage conditions (average content of 15.0% w/w DM), with an increase in the moisture content from 5.7% to 10.9%, which exceeds the specifications ($\leq$ 9.0%). Over the 26-week storage under accelerated conditions, the content of 3-FL (expressed on a DM basis) remained relatively unchanged (average content of 15.1% w/w DM), although an increase from 5.7% to 9.9%, again above the specifications, was observed for the moisture content. Under both normal and accelerated conditions, the total concentration and composition of the HiMO mixture (expressed on a DM basis) remained relatively constant.

The applicant was also requested to provide microbiological analysis, in light of the increase in the moisture content throughout the storage period. Thus, three batches of the NF and three batches of the above-mentioned HiMO mixture stored under warehouse conditions for 25 months were analysed for total viable counts, yeasts and moulds. Microbial levels were below the respective limits of detection and the average moisture content was within the specifications (average content of 8.5 $\pm$ 0.8% in the NF and 6.6 $\pm$ 1.2% in the HiMO mixture).

The applicant also referred to stability studies (e.g. GRN 547, GRN 571, GRN 659 and GRN 833) on other neutral HiMOs such as 2′-FL, LNnT and LNT (EFSA NDA Panel, 2019a,b, 2020c; US FDA, 2015a,b, 2016, 2019). In addition, the stability of the authorised NF has been demonstrated for at least 24 months when stored at room temperature (EFSA NDA Panel, 2021). The applicant proposes a 2-year shelf-life for the NF at 25°C and 60% RH.

The Panel considers that the data provided sufficient information with respect to the stability of the NF for 2 years.

Stability of the NF under the intended conditions of use

A stability study was conducted with two batches of powdered IF and three batches of a ready-to-use liquid IF using the above-mentioned HiMO mixture, which contains the NF. The concentration of the individual HiMOs (HPAEC-PAD) and pH levels were determined immediately after production and after 3- and 6-month storage at ambient conditions. The content of 3-FL remained relatively constant over the storage period and its stability in IF was demonstrated up to 6 months at ambient conditions.

No stability data for 3-FL in other food matrices were provided. The NDA Panel concluded in its previous assessment of this HiMO that 'the data provided sufficient information with respect to the stability of the NF in the food matrices at neutral pH under proper storage conditions. The Panel noted that the acidic pH and especially thermal treatments may decrease the 3-FL content' (EFSA NDA Panel, 2021). Moreover, 2′-FL, the constitutional isomer of 3-FL, has been demonstrated to be stable in various food matrices, including IF, yoghurt, ready-to-drink flavoured milk and citrus fruit beverages (EFSA NDA Panel, 2015a).

The Panel considers that the already available information is sufficient with respect to the stability of the NF in the food matrices.

3.5. Specifications

The specifications of the NF are indicated in Table 3.

Table 3: Specifications of the NF

Description: 3-Fucosyllactose (3-FL) is a white- to ivory-coloured powder produced by microbial fermentation and further isolated, purified and concentrated.

Source: A genetically modified strain of *Escherichia coli* BL21 (DE3).

Parameter	Specification
Composition	
3-FL (% w/w DM)	≥ 90
D-Lactose (% w/w DM)	≤ 5
D-Glucose (% w/w DM)	≤ 3
D-Galactose (% w/w DM)	≤ 3
L-Fucose (% w/w DM)	≤ 3
Sum of other carbohydrates[1] (% w/w DM)	≤ 5
Water (%)	≤ 9.0
Protein (%)	≤ 0.01
Ash (%)	≤ 1.0
Contaminants	
Arsenic (mg/kg)	≤ 0.2
Aflatoxin M1 (μg/kg)	≤ 0.025
Microbiological parameters	
Standard plate count (CFU/g)	≤ 1,000
Yeast and mould (CFU/g)	≤ 100
Enterobacteriaceae (CFU/g)	≤ 10
Salmonella (in 25 g)	ND
Cronobacter (*Enterobacter*) sakazakii (in 10 g)	ND
Endotoxins (EU/mg)	≤ 10

3-FL: 3-Fucosyllactose; CFU: Colony forming unit; DM: Dry matter; EU: Endotoxin unit; ND: Not detected.
(1): Sum of other carbohydrates = 100 (% w/w DM) – 3-FL (% w/w DM) – Quantified carbohydrates (% w/w DM) – Ash (% w/w DM). For those batches of the NF where the levels of any carbohydrate by-product were below the respective limit of quantification (LOQ), the concentration of the corresponding compound has been considered to be equal to the respective LOQ value for the purpose of calculating the sum of other carbohydrates.

The Panel considers that the information provided on the specifications of the NF is sufficient and does not raise safety concerns.

3.6. History of use of the NF and/or of its source

3.6.1. History of use of the NF

There is no history of use of the NF. However, 3-FL produced by fermentation with a derivative strain of *E. coli* K-12 MG1655 has been previously assessed by EFSA as a NF (EFSA NDA Panel, 2021) and authorised in the European Union (Commission Implementing Regulation (EU) 2021/2029[10]).

The history of human exposure to 3-FL is limited primarily to that of breastfed infants. 3-FL was found in the human milk of most women (> 96%) in 10 countries studied (Erney et al., 2000, 2001). The Panel also notes that 3-FL is among the most represented HMOs and shows increasing levels over the course of lactation (Thurl et al., 2017; Samuel et al., 2019). Oligosaccharides in bovine milk are more than 20 times less concentrated than in human milk and the vast majority (~ 90%) is composed of acidic oligosaccharides (Bode, 2012; Aldredge et al., 2013; Albrecht et al., 2014). Wang et al. (2020) have reported mean concentrations of 3-FL of 0.09 and 0.07 g/L in bovine milk and goat milk, respectively.

[10] Commission Implementing Regulation (EU) 2021/2029 of 19 November 2021 authorising the placing on the market of 3-Fucosyllactose (3-FL) as a novel food under Regulation (EU) 2015/2283 of the European Parliament and of the Council and amending Commission Implementing Regulation (EU) 2017/2470; OJ L 415, 22.11.2021, p. 9–14.

3.6.2. Intake of 3-FL from human milk

As reported in previous EFSA opinions (EFSA NDA Panel, 2019b, 2020a,b, 2021), human milk contains a family of structurally related oligosaccharides, known as HMOs, which is the third largest fraction of solid components. The highest concentrations of HMOs occur in human colostrum (20–25 g/L), and concentrations between 5 and 20 g/L occur in mature human milk (Thurl et al., 2010; Bode, 2012; Gidrewicz and Fenton, 2014; Urashima et al., 2018). HMOs concentrations and composition vary across mothers and over the course of lactation. 3-FL belongs to the subfraction of 'neutral core' HMOs, characterised by the presence of N-acetyl-D-glucosamine (GlcNAc) or L-fucose. This fraction accounts for up to 80% of the total HMO concentration (Thurl et al., 2010; Rijnierse et al., 2011; Bode, 2012).

Several publications on HMOs and 3-FL show a wide variability of concentrations in human milk. From the systematic review by Thurl et al. (2017), the reported range of mean concentrations of 3-FL in human milk is 0.24–1.24 g/L for 0–100 days lactation, with maximum means of 0.34–1.44 g/L, and increasing concentrations over time. In another study (Erney et al., 2001), an average concentration of 1.39 g/L and a maximum concentration of 3.92 g/L for 3-FL have been reported.

Other publications reported maximum concentrations in European human milks up to 3.4 g 3-FL/L (average 0.2–2.3 g/L; Austin et al., 2019) or up to 5.7 g 3-FL/L (average 0.4–1.2 g/L; Samuel et al., 2019). In a recent review (Soyyılmaz et al., 2021), a mean of mean concentrations and a maximum mean of 0.92 and 2.57 g 3-FL/L, respectively, were recorded in mature milk (> 90 days).

In consideration of the large and recent data set used in this review (Soyyılmaz et al., 2021), the Panel decided to use the values corresponding to the mean of means (0.92 g/L) and the maximum mean (2.57 g/L) as representative of the average natural concentrations found in human milk.

Based on these reported concentrations of 3-FL in human milk and considering the average and high daily intakes of human milk (800 mL and 1,200 mL, respectively) for infants from 0 to 6 months (EFSA NDA Panel, 2013), the daily intake levels of 3-FL from human milk for a 6.7-kg body weight (bw) infant (EFSA Scientific Committee, 2012) have been calculated (Table 4). This default body weight used by the NDA Panel is for an infant of 3–6 months of age, who is more likely than younger infants to consume these volumes of human milk.

Table 4: Estimated daily intake levels of 3-FL from average (800 mL) and high (1,200 mL) human milk intake for infants of 6.7 kg bw, based on mean and high concentrations of 3-FL of 0.92 g/L and 2.57 g/L, respectively, in mature human milk (lactation day > 90) (Soyyılmaz et al., 2021)

	Daily intake levels (mg/kg bw) from 800 mL of human milk		**Daily intake levels (mg/kg bw) from 1,200 mL of human milk**	
	Mean concentration	**High concentration**	**Mean concentration**	**High concentration**
3-FL	110	307	165	460

bw: body weight.

3.7. Proposed uses and use levels and anticipated intake

3.7.1. Target population

The target population proposed by the applicant is the general population.

3.7.2. Proposed uses and use levels

The NF is proposed to be used as an ingredient in IF and FOF, processed cereal-based food and baby food for infants and young children and milk-based drinks and similar products intended for young children. These food products, defined using the FoodEx2[11] hierarchy, and the maximum use levels are reported in Table 5.

The applicant also intends to market the NF in FS, at the maximum daily intake of 3.0 g/day for individuals above 3 years of age or at a maximum daily intake of 1.2 g/day when intended for infants (up to 11 months) or young children (12–35 months).

[11] FoodEx2 is an EFSA standardised food classification and description system https://www.efsa.europa.eu/en/data/data-standardisation

For the category FSMP, the applicant proposed the use in accordance with the particular nutritional requirements of the persons for whom the products are intended according to Regulation (EU) No 609/2013.

According to the applicant, FS are not intended to be used if other foods with added NF or human milk are consumed on the same day.

Table 5: Food categories according to FoodEx2 hierarchy and maximum use levels of the NF intended by the applicant

FoodEx2 code	FoodEx2 level	Food category	Max use level (mg/100 g)
A03PZ	4	Infant formulae, powder	720[a]
A03QE	4	Infant formulae, liquid	90
A03QK	4	Follow-on formulae, powder	960[a]
A03QQ	4	Follow-on formulae, liquid	120
A03QZ	3	Cereals with an added high protein food which have to be reconstituted with water or other protein-free liquid	480[a]
A03QY	3	Simple cereals which have to be reconstituted with milk or other appropriate nutritious liquids	840[a]
A0BZF	3	Cereals with an added high protein food reconstituted	120
A0BZE	3	Simple cereals for infants and children, reconstituted	120
A03RA	3	Biscuits, rusks and cookies for children	120
A03RB	3	Pasta for children (dry, to be cooked)	120
A03RH	3	Ready-to-eat dairy-based meal for children	120
A03RP	3	Special food for children's growth	120
A03RN	3	Fruit and vegetable juices and nectars specific for infants and young children	120

(a): Relevant dilution factors (EFSA, 2018) have been used to calculate intake estimates applying the FoodEx2 food classification and description system.

3.7.3. Anticipated intake of the NF

Anticipated intake of 3-FL from the proposed use level of the NF in IF in infants up to 16 weeks of age

IF is expected to be the only food consumed by infants aged 0–16 weeks who are not breastfed. A high consumption of IF has been estimated to be 260 mL/kg bw per day for infants aged 0–16 weeks (EFSA Scientific Committee, 2017). Based on the maximum proposed use level of the NF (0.9 g/L in IF), the high intake of the NF from IF alone is estimated to be 234 mg/kg bw per day.

The Panel notes that the anticipated daily intake of the NF from the consumption of IF does not exceed the estimated high daily intake of 3-FL of 460 mg/kg bw in breastfed infants (Table 4).

Anticipated intake of 3-FL from the proposed uses and use levels of the NF

EFSA performed an assessment of the anticipated daily intake of the NF based on the applicant's proposed uses and maximum proposed use levels (Table 5), using individual data from the EFSA Comprehensive European Food Consumption Database (EFSA, 2011). The lowest and highest mean and 95th percentile anticipated daily intakes of the NF (on a mg/kg bw basis), among the EU dietary surveys, are presented in Table 6.

The estimated daily intake of the NF for each population group from each EU dietary survey is available in the Excel file annexed to this scientific opinion (under supporting information).

Table 6: Intake estimate resulting from the use of 3-FL as an ingredient in the intended food categories at the maximum proposed use levels

Population group	Age (years)	Mean intake (mg/kg bw per day)		P95th intake (mg/kg bw per day)	
		Lowest[(a)]	Highest[(a)]	Lowest[(b)]	Highest[(b)]
Infants	< 1	22	100	80	199
Young children[(c)]	1 to < 3	1	40	13	96
Other children	3 to < 10	0	2	0	13
Adolescents	10 to < 18	0	1	0	3
Adults[(d)]	≥ 18	0	0	0	1

(a): Intakes were assessed for all EU dietary surveys available in the food comprehensive database on 15 March 2022. The lowest and the highest averages observed among all EU surveys are reported in these columns.
(b): Intakes were assessed for all EU dietary surveys available in the food comprehensive database on 15 March 2022. The lowest and the highest P95th observed among all EU surveys are reported in these columns (P95th based on less than 60 individuals are not considered).
(c): Referred as 'toddlers' in the EFSA food consumption comprehensive database (EFSA, 2011).
(d): Includes elderly, very elderly, pregnant and lactating women.

The Panel notes that the content of 3-FL in the NF accounts for about 96.5%; therefore, the figures that are calculated considering a 100% purity slightly overestimate the actual intake. The Panel also notes that the anticipated daily intake of the NF from the proposed uses and use levels does not exceed the estimated high daily intake of 460 mg/kg bw of 3-FL in breastfed infants (Table 4).

3.7.4. Anticipated use of the NF from food supplements

The applicant has proposed a maximum use level of the NF of 3.0 g/day in FS for individuals above 3 years of age or 1.2 g/day when intended for infants (up to 11 months) or young children (12–35 months).

Table 7: Use of the NF in FS and resulting intake expressed as mg/kg bw per day

Population group	Age (years)	Body weight[(a)] (kg)	Use level (mg/day)	Intake (mg/kg bw per day)[(b)]
Infants	< 1	5.0	1,200	240
Young children[(c)]	1 to < 3	12.0	1,200	100
Other children	3 to < 10	23.1	3,000	130
Young adolescents	10 to < 14	43.4	3,000	69
Old adolescents	14 to < 18	61.3	3,000	49
Adults	≥ 18	70.0	3,000	43

(a): Default and average body weights for each population group are available in EFSA Scientific committee (2012).
(b): Intake in 'mg/kg bw per day' is calculated by considering the use levels in 'mg/day' and default body weights defined in EFSA Scientific committee (2012).
(c): Referred as 'toddlers' in the EFSA food consumption comprehensive database (EFSA, 2011).

The Panel notes that the maximum daily intake of the NF from its use in FS (i.e. from 43 to 240 mg/kg bw per day) for any population category (Table 7) does not exceed the estimated high daily intake of 3-FL of 460 mg/kg bw in breastfed infants (Table 4).

According to the applicant, FS containing the NF are not intended to be used if other foods with added 3-FL are also consumed on the same day. The Panel similarly notes that infants and young children should not consume human milk and the FS on the same day.

3.7.5. Combined intake from the NF and other sources

The Panel notes that 3-FL is already authorised for use in food categories other than those proposed for the NF under assessment (e.g. use in beverages, flavoured and unflavoured fermented milk-based products, cereal bars).[4]

The combined daily intake of 3-FL from the authorised and proposed uses, for each population group from each EU dietary survey, is available in the Excel file annexed to this scientific opinion (under supporting information).

Therefore, the combined intake of 3-FL from already authorised uses and the currently proposed uses is higher (highest P95th intake of 366 mg/kg bw in infants, Table 8) than the estimated intake based on only the currently proposed uses and use levels (highest P95th intake of 199 mg/kg bw in infants, Table 6).

Table 8: Intake estimate resulting from the combined uses of 3-FL from both authorised and proposed food categories at the maximum use levels

Population group	Age (years)	Mean intake (mg/kg bw per day)		P95th intake (mg/kg bw per day)	
		Lowest[(a)]	Highest[(a)]	Lowest[(b)]	Highest[(b)]
Infants	< 1	35	149	90	366
Young children[(c)]	1 to < 3	31	104	82	254
Other children	3 to < 10	12	49	31	119
Adolescents	10 to < 18	4	16	16	38
Adults[(d)]	≥ 18	6	10	20	24

(a): Intakes were assessed for all EU dietary surveys available in the food comprehensive database on 15 March 2022. The lowest and the highest averages observed among all EU surveys are reported in these columns.
(b): Intakes were assessed for all EU dietary surveys available in the food comprehensive database on 15 March 2022. The lowest and the highest P95th observed among all EU surveys are reported in these columns (P95th based on less than 60 individuals are not considered).
(c): Referred as 'toddlers' in the EFSA food consumption comprehensive database (EFSA, 2011).
(d): Includes elderly, very elderly, pregnant and lactating women.

The Panel notes that the highest estimated 95th percentile daily intake in infants from the combined exposure (i.e. 366 mg/kg bw) from the maximum authorised and proposed uses, is similar to the estimated intake from the authorised uses alone (i.e. 361 mg/kg bw; EFSA NDA Panel, 2021), and below the high estimate for 3-FL intake from human milk (i.e. 460 mg/kg bw; Table 4).

3.8. Absorption, distribution, metabolism and excretion (ADME)

No ADME data have been provided for the NF.

As reported in previous EFSA opinions (EFSA NDA Panel, 2014, 2019a,b, 2020a,b, 2021) HMOs, including 3-FL, are considered 'non-digestible oligosaccharides' since they do not undergo any significant digestion in the upper gastrointestinal tract (Brand-Miller et al., 1995, 1998; Engfer et al., 2000; Chaturvedi et al., 2001; Gnoth et al., 2001; Rudloff and Kunz, 2012)

Brand-Miller et al. (1995, 1998) reported that HMOs, consumed as a load (a purified oligosaccharide fraction from human milk), are fermented in the colon by the intestinal microbiota. Chaturvedi et al. (2001) and Coppa et al. (2001) reported that 97% and 40–50%, respectively, of the ingested HMOs are excreted unchanged in faeces of breastfed infants. Furthermore, approximately 1–2% of the ingested amounts of HMOs is excreted unchanged in the infants' urine (Rudloff et al., 1996, 2006; Gnoth et al., 2000; Goehring et al., 2014; Vazquez et al., 2017; EFSA NDA Panel, 2019b).

In addition, Gnoth et al. (2001) have suggested that small quantities of 3-FL may be transported transcellularly across the intestinal epithelium by receptor-mediated transcytosis, and/or by paracellular means, and low quantities of unchanged 3-FL have been detected in the urine of breastfed infants (Rudloff et al., 1996, 2012; Obermeier et al., 1999; Chaturvedi et al., 2001; Dotz et al., 2014).

Based on information available on HMOs, the Panel considers that limited digestion of the NF occurs in the gastrointestinal tract and that only small amounts are expected to be absorbed. Moreover, there are no indications that the absorption of 3-FL, which is the main constituent of the NF or other structurally related mono- and oligosaccharides (e.g. D-lactose), differs from that of similar components found in human milk (EFSA NDA Panel, 2021).

3.9. Nutritional information

The NF is mainly composed of the non-digestible oligosaccharide 3-FL.

The Panel considers that consumption of the NF under the proposed conditions of use is not nutritionally disadvantageous.

3.10. Toxicological information

The applicant provided three toxicological studies on a mixture of HiMOs containing the NF, which were conducted in compliance with OECD (Organisation for Economic Cooperation and Development) principles of Good Laboratory Practices (GLP) (OECD, 1998) and in accordance with the OECD test guidelines (TG) 471 (OECD, 1997), 487 (OECD, 2016) and 408 (OECD, 2018). An additional preliminary *in vivo* repeated dose study was also carried out. These studies were conducted with a mixture of HiMOs composed by 2′-FL (47.1%), 3-FL (16.0%), LNT (23.7%), 3′-SL sodium salt (4.1%), 6′-SL sodium salt (4.0%) and other carbohydrates (5.1%). These studies, which were claimed proprietary by the applicant, are listed in Table 9.

An article on the assessment of the NF in the above-mentioned mixture of HiMOs, which describes the studies listed in Table 9, was provided (Parschat et al., 2020).

Table 9: List of toxicological studies with the NF (as component of the mixture of HiMOs)

Reference	Type of study	Test system	Dose
Unpublished Study, LPT No. 35908 (Parschat et al., 2020)	Bacterial reverse mutation test (GLP, OECD TG 471)	*Salmonella* Typhimurium TA98, TA100, TA102, TA1535 and TA1537	0.8–90 mg 3-FL/plate (absence and presence of S9 mix)
Unpublished Study, LPT No. 35909 (Parschat et al., 2020)	*In vitro* mammalian cell micronucleus test in human peripheral blood lymphocytes (GLP, OECD TG 487)	Human peripheral blood lymphocytes	1.2–9.6 mg 3-FL/mL for 4 or 24 h (absence and presence of S9 mix)
Unpublished study, LPT No. 35504 (Parschat et al., 2020)	7-day repeated dose oral toxicity study (pilot study)	SD rats (females only)	Dietary exposure ranging from 6.7 to 13.7 g/kg bw/day (mean 3-FL intake: 1.07–2.19 g/kg bw/day)
Unpublished Study, LPT No. 35907 (Parschat et al., 2020)	90-day repeated dose oral toxicity study (GLP, OECD TG 408, limit test)	SD rats	Overall dietary exposure to 3-FL of 16% of the mixture (mean intake of 0.91 and 1.12 g 3-FL/kg bw/day in males and females, respectively)

3.10.1. Genotoxicity

The *in vitro* assessment of the mutagenic potential of the mixture of HiMOs containing the NF was performed with *S*. Typhimurium strains TA98, TA100, TA102, TA1535 and TA1537, which were exposed to the mixture diluted in water at six different concentrations up to 600 mg mixture/plate, either in the presence or absence of liver microsomal fractions (S9). No reproducible or dose-related increases in revertant colony numbers over control counts were observed with any of the strains following exposure to the mixture at any concentration (irrespective of the presence or absence of S9). No evidence of toxicity was obtained following exposure to the mixture of HiMOs. Therefore, the mixture of HiMOs was shown to be non-mutagenic at concentrations up to 600 mg/plate (corresponding to about 96 mg/plate of 3-FL), in the absence or presence of metabolic activation.

In the *in vitro* mammalian cell micronucleus test, five concentrations of the mixture of HiMOs up to 60 mg/mL were tested in cultured human peripheral blood lymphocytes in the presence or absence of metabolic activation (S9 fraction). No statistically significant increases in the number of binucleated cells containing micronuclei both after 4-h treatment in the presence of S9 mix or following 24-h treatment in the absence of S9 was recorded. The mixture of HiMOs did not show any evidence of clastogenicity or aneugenicity in the absence and presence of metabolic activation up to the highest concentration of 60 mg/mL (corresponding to about 10 mg 3-FL/mL).

Taking into account the results provided and considering the nature, source and production process of the NF, the Panel considers that there are no concerns regarding genotoxicity.

3.10.2. Repeated dose toxicity studies

The applicant provided a 7-day repeated dose pilot toxicity study, where two groups of five Crl:CD (SD) female rats were given *ad libitum* a standard diet with and without 10% (w/w) of mixture of HiMOs. The calculated intake of the mixture ranged from 6.7 to 13.7 g/kg bw per day, corresponding to a 3-FL intake of 1.07–2.19 g/kg bw per day. There were no deaths or any relevant variations in clinical signs, food consumption or body weight. Clinical pathology investigations and post-mortem observations were not performed.

In the 90-day study (limit test) groups of 10 Crl:CD(SD) rats/sex were given *ad libitum* a standard diet with or without 10% (w/w) of the mixture of HiMOs (same composition as in the pilot study). The mean intake of the test item ranged from 5.01 to 6.88 g/kg bw per day (mean of 5.67) for the male animals and from 6.26 to 7.91 g/kg bw per day (mean of 6.97) for the female animals. The corresponding mean daily intake of 3-FL has been calculated as 0.91 and 1.12 g/kg bw for male and female rats, respectively.

There were no deaths in the course of the study and no treatment-related changes in clinical signs were observed in any rats. Episodes of increased or decreased food consumption were recorded in treated males in comparison to the control group. Body weight and body weight gain were not affected by the treatment. Some statistically significant changes were noted: reduced spontaneous motility was observed in treated male rats in the absence of any other change in functional observation tests and a slight increase in body temperature was noted in female rats.

Variations in haematological (decrease of neutrophils (−11%) in females) and clinical chemistry parameters (decrease in proteins (−9%, both albumin and globulin and increase in albumin/globulin ratio) and alanine aminotransferase (−24%), increase in urea (+16%) in females) and urinalysis (decrease in specific gravity (−1%) in females) were recorded. A decrease in absolute brain weight (−2.9%) in treated males and relative kidneys weight (about −10%) in female rats was also noted. In male animals at histological examination, a small increase in the incidence and magnitude of hepatocellular lipid content in periportal areas was recorded. No other gross or histopathologic findings in treated rats were noted.

The changes observed were of low magnitude and limited to only one sex and they are overall considered by the Panel as not biologically relevant.

The Panel considers that no adverse effects were observed in this study at the tested dose corresponding to 0.91 g 3-FL/kg bw per day.

3.10.3. Human data

No human intervention studies with 3-FL alone have been provided by the applicant.

The Panel noted that a recent publication (Parschat et al., 2021) refers to a double-blind, controlled, randomised interventional study conducted in infants with IF containing the mixture of HiMOs described in Section 3.10.2 (5.75 g/L, corresponding to about 0.9 g/L of 3-FL). The main goal of the study was to investigate the suitability of the HiMO mixture – IF to support normal physical growth (evaluated per weight gain), in comparison with standard IF and breastfed infants. The study was conducted over a 112-day period in a total of 341 subjects. Secondary endpoints of tolerability (e.g. stool frequency and consistency, digestive tolerance) were also assessed. The safety and tolerability profile of the HiMO mixture – IF appeared similar to the commercialised IF alone used as a comparator.

In addition, the applicant referred to a few human studies which have been performed with the constitutional isomer 2′-FL. In these randomised, double-blind, controlled intervention studies in infants, 2′-FL was administered at concentrations ranging from 0.2 to 1.0 g 2′-FL/L alone or in combination with LNnT or other oligosaccharides (e.g. galacto-oligosaccharides (GOS)) (Marriage et al., 2015; Kajzer et al., 2016; Puccio et al., 2017). According to the authors, the available data were overall sufficient to conclude about the safety of 2′-FL under the proposed conditions of use. In addition, the study by Elison et al. (2016), which was previously assessed by the NDA Panel (EFSA NDA Panel, 2015a,c, 2019b), reported a statistically significant increase in gastrointestinal symptoms (e.g. nausea, bloating, loose stools) in adults consuming 20 g/day of 2′-FL for 2 weeks as compared with the placebo group.

The Panel considers the information provided by the applicant as supportive for the assessment of 3-FL.

3.11. Allergenicity

The protein content in the NF is low (≤ 0.01%) as indicated in the specifications (Table 3).

The applicant provided evidence for the absence of viable cells of the production strain in the NF.

The applicant did not identify any allergenic potential of introduced proteins as a result of the genetic modification of the *E. coli* BL21 (DE3) host strain according to the 'Scientific opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed of the Scientific Panel on Genetically Modified Organisms' (EFSA GMO Panel, 2010), using 'higher than 35% identity in a sliding window of 80 amino acids' as the criterion.

The Panel considers that, for these reasons, the likelihood of allergenic reactions to the NF is low.

4. Discussion

The NF is a powdered mixture mainly composed of the HiMO 3-FL, but it also contains D-lactose, D-glucose, D-galactose and L-fucose, and a small fraction of other related saccharides. The NF is obtained by fermentation with a genetically modified strain of *E. coli* BL21 (DE3).

The applicant intends to add the NF to a variety of foods, including IF and FOF, food intended for infants and young children, FSMP and FS. The target population proposed by the applicant is the general population.

Considering that 3-FL is a naturally occurring oligosaccharide present in human milk, the history of human exposure to 3-FL concerns breastfed infants. The intake of 3-FL in breastfed infants on a bw basis is expected to be safe also for other population groups.

The Panel notes that a safety assessment of 3-FL, when produced by a derivative strain of *E. coli* K-12 MG1655, has been carried out by EFSA (EFSA NDA Panel, 2021) and 3-FL is included in the Union list of authorised NFs. The Panel also notes that other HiMOs (LNnT and LNT) produced by fermentation with derivative strains of the same host strain *E. coli* BL21 (DE3) have been recently assessed with a positive outcome (EFSA NDA Panel, 2020c, 2022c).

The submitted toxicity studies did not raise safety concerns. The Panel considers that no adverse effects were observed in the subchronic toxicity study at the tested dose corresponding to a daily intake of 0.91 g 3-FL/kg bw.

The Panel notes that the anticipated daily intake of 3-FL from the consumption of IF (only), in infants up to 16 weeks of age, does not exceed the highest intake level of 3-FL in breastfed infants on a bw basis. The anticipated daily intake of 3-FL from both proposed and combined (authorised and proposed) uses at their respective maximum use levels in all population categories was also not above the highest intake level of 3-FL from human milk in infants on a bw basis.

The maximum daily intake of 3-FL in FS for individuals above 3 years of age (i.e. 3 g/day) or in infants and young children (i.e. 1.2 g/day) does not exceed the highest intake level of 3-FL in breastfed infants per kg bw. The applicant stated that FS containing the NF are not intended to be used if other foods with added NF are consumed on the same day. The Panel similarly notes that infants and young children should not consume human milk and the FS on the same day.

Taking into account the intrinsic nature of HMOs with their limited absorption, the absence of toxicologically relevant effects in the subchronic study and considering that infants are naturally exposed to these substances, the Panel considers that the consumption of the NF at the proposed uses and use levels does not raise safety concerns.

Finally, it is noted that, in line with other milk oligosaccharides that are natural components of human milk, the safety assessment of this NF is mainly based on the comparison between the intake of breastfed infants and the estimated intake as NF.

5. Conclusions

The Panel concludes that the NF, which is composed of 3-FL and other structurally related mono- and oligosaccharides, is safe under the proposed conditions of use.

5.1. Protection of Proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283

The Panel could not have reached the conclusion on the safety of the NF under the proposed conditions of use without the data claimed as proprietary by the applicant: (i) identity of the NF as confirmed by MS, NMR spectroscopy and HPAEC-PAD; (ii) toxicological information, including *in vitro* genotoxicity studies and the subchronic toxicity study (Table 9); (iii) description of the genetically modified production strain, qPCR detection system and method validation report, and certificate of deposition of the production strain; (iv) method validation reports for the determination of 3-FL and carbohydrate by-products in the NF using HPAEC-PAD.

6. Steps taken by EFSA

1) On 23 September 2020 EFSA received a letter from the European Commission with the request for a scientific opinion on the safety of 3-fucosyllactose (3-FL). Ref. Ares(2020) 4973960 – 23/09/2020.
2) On 23 September 2020, a valid application on 3-fucosyllactose (3-FL), which was submitted by Chr. Hansen A/S, was made available to EFSA by the European Commission through the Commission e-submission portal (NF 2020/1620) and the scientific evaluation procedure was initiated.
3) On 14 December 2020, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4) On 11 November 2021, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
5) During its meeting on 29 April 2022, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of 3-fucosyllactose as a NF pursuant to Regulation (EU) 2015/2283.

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.
Austin S, De Castro CA, Sprenger N, Binia A, Affolter M, Garcia-Rodenas CL, Beauport L, Tolsa JF and Fischer Fumeaux CJ, 2019. Human milk oligosaccharides in the milk of mothers delivering term versus preterm infants. Nutrients, 11, 1282.
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. The Journal of Pediatrics, 133, 95–98.
Chart H, Smith HR, La Ragione RM and Woodward MJ, 2000. An investigation into the pathogenic properties of *Escherichia coli* strains BLR, BL21, DH5alpha and EQ1. Journal of Applied Microbiology, 89, 1048–1058.
Chaturvedi P, Warren CD, Buescher CR, Pickering LK and Newburg DS, 2001. Survival of human milk oligosaccharides in the intestine of infants. In: Newberg DS (ed.). Bioactive components of human milk. (Advances in experimental medicine and biology, volume 501). Springer Science+Business Media, New York, pp. 315–323.
Choi SS, Lynch BS, Baldwin N, Dakoulas EW, Roy S, Moore C, Thorsrud BA and Rohrig CH, 2015. Safety evaluation of the human-identical milk monosaccharide, L-fucose. Regulatory Toxicology and Pharmacology, 72, 39–48.
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.
Dotz V, Rudloff S, Blank D, Lochnit G, Geyer R and Kunz C, 2014. 13C-labeled oligosaccharides in breastfed infants' urine: individual-, structure- and time-dependent differences in the excretion. Glycobiology, 24, 185–194.
EFSA (European Food Safety Authority), 2011. Use of the EFSA Comprehensive European Food Consumption Database in exposure assessment. EFSA Journal 2011;9(3):2097, 34 pp. https://doi.org/10.2903/j.efsa.2011.2097
EFSA (European Food Safety Authority), Arcella D, Ioannidou S and Sousa R, 2018. Internal report on the harmonisation of dilution factors to be used in the assessment of dietary exposure. EFSA Journal 2018. https://doi.org/10.5281/zenodo.1256085
EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2018. Guidance on the characterisation of microorganisms used as feed additives or as production organisms. EFSA Journal 2018;16(3):5206, 24 pp. https://doi.org/10.2903/j.efsa.2018.5206
EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010. Scientific opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. EFSA Journal 2010;8(7):1700, 168 pp. https://doi.org/10.2903/j.efsa.2010.1700

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2013. Scientific opinion on nutrient requirements and dietary intakes of infants and young children in the European Union. EFSA Journal 2013;11(10):3408, 103 pp. https://doi.org/10.2903/j.efsa.2013.3408

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. https://doi.org/10.2903/j.efsa.2014.3760

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2015a. 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. https://doi.org/10.2903/j.efsa.2015.4184

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2015b. 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. https://doi.org/10.2903/j.efsa.2015.4183

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2015c. Statement on the safety of lacto-N-neotetraose and 2′-O-fucosyllactose as novel food ingredients in food supplements for children. EFSA Journal 2015;13(11):4299, 11 pp. https://doi.org/10.2903/j.efsa.2015.4299

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2016. Guidance on the preparation of an application for authorisation of a novel food in the context of Regulation (EU) 2015/2283. EFSA Journal 2016;14(11):4594, 24 pp. https://doi.org/10.2903/j.efsa.2016.4594

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2017. Scientific opinion on the safety of synthetic N-acetyl-D-neuraminic acid as a novel food pursuant to Regulation (EC) No 258/97. EFSA Journal 2017;15(7):4918, 28 pp. https://doi.org/10.2903/j.efsa.2017.4918

EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens), 2019a. Scientific opinion on the safety of lacto-N-tetraose (LNT) as a novel food pursuant to Regulation (EU) 2015/2283. EFSA Journal 2019;17(12):5907, 27 pp. https://doi.org/10.2903/j.efsa.2019.5907

EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens), 2019b. Scientific opinion on the safety of 2′-fucosyllactose/difucosyllactose mixture as a novel food pursuant to Regulation (EU) 2015/2283. EFSA Journal 2019;17(6):5717, 23 pp. https://doi.org/10.2903/j.efsa.2019.5717

EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens), 2020a. Scientific opinion on the safety of 3′-Sialyllactose (3′-SL) sodium salt as a novel food pursuant to Regulation (EU) 2015/2283. EFSA Journal 2020;18(5):6098, 22 pp. https://doi.org/10.2903/j.efsa.2020.6098

EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens), 2020b. Scientific opinion on the safety of 6′-Sialyllactose (6′-SL) sodium salt as a novel food pursuant to Regulation (EU) 2015/2283. EFSA Journal 2020;18(5):6097, 23 pp. https://doi.org/10.2903/j.efsa.2020.6097

EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens), 2020c. Scientific opinion on the safety of lacto-N-neotetraose (LNnT) produced by derivative strains of E. coli BL21 as a novel food pursuant to Regulation (EU) 2015/2283. EFSA Journal 2020;18(11):6305, 11 pp. https://doi.org/10.2903/j.efsa.2020.6305

EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens), 2021. Scientific opinion on the safety of 3-FL (3-Fucosyllactose) as a novel food pursuant to Regulation (EU) 2015/2283. EFSA Journal 2021;19(6):6662, 25 pp. https://doi.org/10.2903/j.efsa.2021.6662

EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens), 2022a. Scientific opinion on the safety of the extension of use of 2′-fucosyllactose/difucosyllactose (2′-FL/DFL) mixture and lacto-N-tetraose (LNT) as novel foods in food supplements for infants pursuant to Regulation (EU) 2015/2283. EFSA Journal 2022;20(3):7140, 9 pp. https://doi.org/10.2903/j.efsa.2022.7140

EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens), 2022b. Scientific Opinion on the safety of the extension of use of 2′-fucosyllactose (2′-FL) and lacto-N-neotetraose (LNnT) as novel foods in food supplements for infants pursuant to Regulation (EU). 2015/2283. EFSA Journal 2022;20(5):7257, 9 pp. https://doi.org/10.2903/j.efsa.2022.7257

EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens), 2022c. Scientific Opinion on the safety of lacto-N-tetraose (LNT) produced by derivative strains of *Escherichia coli* BL21 (DE3) as a Novel Food pursuant to Regulation (EU) 2015/2283. EFSA Journal 2022;20(5):7242, 24 pp. https://doi.org/10.2903/j.efsa.2022.7242

EFSA Scientific Committee, 2012. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 2012;10(3):2579, 32 pp. https://doi.org/10.2903/j.efsa.2012.2579

EFSA Scientific Committee, 2017. Guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age. EFSA Journal 2017;15(5):4849, 58 pp. https://doi.org/10.2903/j.efsa.2017.4849

Elison E, Vigsnaes LK, Rindom Krogsgaard L, Rasmussen J, Sørensen N, McConnell B, Hennet T, Sommer MO and Bytzer P, 2016. Oral supplementation of healthy adults with 2′-O-fucosyllactose and lacto-N-neotetraose is well tolerated and shifts the intestinal microbiota. British Journal of Nutrition, 116, 1356–1368.

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. The American Journal of Clinical Nutrition, 71, 1589–1596.

Erney R, Hilty M, Pickering L, Ruiz-Palacios G and Prieto P, 2001. Human milk oligosaccharides: a novel method provides insight into human genetics. In: Newburg DS (eds.), Bioactive Components of Human Milk. Advances in Experimental Medicine and Biology, vol 501, pp. 285–297. Springer, Boston, MA. https://doi.org/10.1007/978-1-4615-1371-1_36

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.

Gidrewicz DA and Fenton TR, 2014. A systematic review and meta-analysis of the nutrient content of preterm and term breast milk. BioMedCentral Pediatrics, 14, 216.

Gnoth MJ, Kunz C, Kinne-Saffran E and Rudloff S, 2000. Human milk oligosaccharides are minimally digested in vitro. The 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.

Goehring KC, Kennedy AD, Prieto PA and Buck RH, 2014. Direct evidence for the presence of human milk oligosaccharides in the circulation of breastfed infants. PLoS One, 9, e101692.

Jeong H, Barbe V, Lee CH, Vallenet D, Yu DS, Choi SH, Couloux A, Lee SW, Yoon SH, Cattolico L, Hur CG, Park HS, Ségurens B, Kim SC, Oh TK, Lenski RE, Studier FW, Daegelen P and Kim JF, 2009. Genome sequences of *Escherichia coli* B strains REL606 and BL21(DE3). Journal of Molecular Biology, 394, 644–652.

Kailemia MJ, Ruhaak LR, Lebrilla CB and Amster IJ, 2014. Oligosaccharide analysis by mass spectrometry: a review of recent developments. Analytical Chemistry, 86, 196–212.

Kajzer J, Oliver J and Marriage B, 2016. Gastrointestinal tolerance of formula supplemented with oligosaccharides. The FASEB Journal, 30, 671.4.

Mank M, Welsch P, Heck AJR and Stahl B, 2019. Label-free targeted LC-ESI-MS2 analysis of human milk oligosaccharides (HMOS) and related human milk groups with enhanced structural selectivity. Analytical and Bioanalytical Chemistry, 411, 231–250.

Marriage BJ, Buck RH, Goehring KC, Oliver JS and Williams JA, 2015. Infants fed a lower calorie formula with 2'-FL show growth and 2'-FL uptake like breast-fed infants. Journal of Pediatric Gastroenterology and Nutrition, 61, 649–658.

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

OECD (Organisation for Economic Co-operation and Development), 1997. 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), 1998. OECD Principles on Good Laboratory Practice, OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, No. 1, OECD Publishing, Paris. https://doi.org/10.1787/9789264078536-en

OECD (Organisation for Economic Co-operation and Development), 2016. Test No. 487: *In vitro* mammalian cell micronucleus test, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris. https://doi.org/10.1787/9789264264861-en

OECD (Organisation for Economic Co-operation and Development), 2018. Test No. 408: Repeated dose 90-day oral toxicity study in rodents, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris. https://doi.org/10.1787/9789264070707-en

Parschat K, Melsaether C, Jäpelt KR and Jennewein S, 2021. Clinical evaluation of 16-week supplementation with 5HMO-mix in healthy-term human infants to determine tolerability, safety, and effect on growth. Nutrients, 13, 2871. https://doi.org/10.3390/nu13082871

Parschat K, Oehme A, Leuschner J, Jennewein S and Parkot J, 2020. A safety evaluation of mixed human milk oligosaccharides in rats. Food and Chemical Toxicology, 136, 111118.

Puccio G, Alliet P, Cajozzo C, Janssens E, Corsello G, Sprenger N, Wernimont S, Egli D, Gosoniu L and Steenhout P, 2017. Effects of infant formula with human milk oligosaccharides on growth and morbidity: a randomized multicenter trial. Journal of Pediatric Gastroenterology and Nutrition, 64, 624–631.

Rijnierse A, Jeurink PV, van Esch BCAM, Garssen J and Knippels LMJ, 2011. Food-derived oligosaccharides exhibit pharmaceutical properties. European Journal of Pharmacology, 668, S117–S123.

Rudloff S and Kunz C, 2012. Milk oligosaccharides and metabolism in infants. American Society for Nutrition. Advances in Nutrition, 3, 398S–405S.

Rudloff S, Obermeier S, Borsch C, Pohlentz G, Hartmann R, Brösicke 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, Diekmann L, Egge H and Kunz C, 1996. Urinary excretion of lactose and oligosaccharides in preterm infants fed human milk or infant formula. Acta Paediatrica, 1996, 598–603.

Rudloff S, Pohlentz G, Borsch C, Lentze M and Kunz C, 2012. Urinary excretion of in vivo 13C-labelled milk oligosaccharides in breastfed infants. British Journal on Nutrition, 107, 957–963.

Samuel TM, Binia A, de Castro CA, Thakkar SK, Billeaud C, Agosti M, Al-Jashi I, Costeira MJ, Marchini G, Martínez-Costa C, Picaud JC, Stiris T, Stoicescu SM, Vanpeé M, Domellöf M, Austin S and Sprenge N, 2019. Impact of maternal characteristics on human milk oligosaccharide composition over the first 4 months of lactation in a cohort of healthy European mothers. Scientific Reports, 9, 11767.
Smilowitz JT, O'sullivan A, Barile D, German JB, Lonnerdal B and Slupsky CM, 2013. The human milk metabolome reveals diverse oligosaccharide profiles. The Journal of Nutrition, 143, 1709–1718.
Soyyılmaz B, Mikš MH, Röhrig CH, Matwiejuk M, Meszaros-Matwiejuk A and Vigsnæs LK, 2021. The mean of milk: a review of human milk oligosaccharide concentrations throughout lactation. Nutrients, 13, 2737.
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.
Thurl S, Munzert M, Boehm G, Matthews C and Stahl B, 2017. Systematic review of the concentrations of oligosaccharides in human milk. Nutrition Reviews, 75, 920–933.
Urashima T, Yamaguchi E, Ohshima T, Fukuda K and Saito T, 2018. Chemical structures of oligosaccharides in milk of the raccoon (*Procyon lotor*). Glycoconjugate Journal, 35, 275–286.
US FDA (US Food and Drug Administration), 2015a. No GRN. 547 [Lacto-N-neotetraose; Glycom A/S, Lyngby, Denmark]. In: GRAS Notices. US FDA, Centre for Food Safety and Applied Nutrition, Office of Food Additive Safety, Silver Spring (MD); Available online: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/?set=GRASNotices&id=547&sort=GRN_No&order=DESC&startrow=1&type=basic&search=547
US FDA (US Food and Drug Administration), 2015b. No GRN. 571 [2'-Fucosyllactose; Jennewein Biotechnologie GmgH, Reinbreitbach, Germany]. In: GRAS Notices. US FDA, Centre for Food Safety and Applied Nutrition, Office of Food Additive Safety, Silver Spring (MD). Available online: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/?set=GRASNotices&id=571&sort=GRN_No&order=DESC&startrow=1&type=basic&search=571
US FDA (US Food and Drug Administration), 2016. No GRN. 659 [Lacto-N-neotetraose; Glycom A/S, Lyngby, Denmark]. In: GRAS Notices. US FDA, Centre for Food Safety and Applied Nutrition, Office of Food Additive Safety, Silver Spring (MD). Available online: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/?set=GRASNotices&id=659&sort=GRN_No&order=DESC&startrow=1&type=basic&search=659
US FDA (US Food and Drug Administration), 2019. No GRN. 833 [Lacto-N-tetraose; Glycom A/S, Hørsholm, Denmark]. In: GRAS Notices. US FDA, Centre for Food Safety and Applied Nutrition, Office of Food Additive Safety, Silver Spring (MD). Available online: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/?set=GRASNotices&id=833&sort=GRN_No&order=DESC&startrow=1&type=basic&search=833
Vazquez E, Santos-Fandila A, Buck R, Rueda R and Ramirez M, 2017. Major human milk oligosaccharides are absorbed into the systemic circulation after oral administration in rats. British Journal of Nutrition, 117, 237–247.
Wang X, Yuan ZF, Fan J, Karch KR, Ball LE, Denu JM and Garcia BA, 2016. A novel quantitative mass spectrometry platform for determining protein o-glcnacylation dynamics. Molecular & Cellular Proteomics, 15, 2462–2475.
Wang Y, Gong ZX, Chen Y, Feng Z, Liu P, Zhang P, Wang X, Zhang L and Song L, 2020. Comparative major oligosaccharides and lactose between Chinese human and animal milk. International Dairy Journal, 108, 104727.
Yamagaki T and Makino Y, 2017. Fragmentation of oligosaccharides from sodium adduct molecules depends on the position of n-acetyl hexosamine residue in their sequences in mass spectrometry. Mass Spectrometry (Tokyo), 6, S0073.
Zaia J, 2004. Mass spectrometry of oligosaccharides. Mass Spectrometry Reviews, 23, 161–227.

Abbreviations

1D	Mono-dimensional
2D	Two-dimensional
2'-FL	2'-Fucosyllactose
3-FL	3-Fucosyllactose
3'-SL	3'-Sialyllactose
6'-SL	6'-Sialyllactose
ADME	Absorption, Distribution, Metabolism and Excretion
ASU	Official collection of analysis methods according to § 64 of the German Food and Feed Code (LFGB)
bw	Body weight
CAS	Chemical Abstracts Service
CFU	Colony forming unit
CID	Collision induced decay
COSY	Correlated spectroscopy
Crl:CD(SD) rats	Charles River Laboratories: Caesarean-derived (Sprague Dawley) rats
DEPT	Distortionless enhancement by polarisation transfer
DFL	Difucosyllactose
DIN	German Institute for Standardisation e. V.

DM	Dry matter
DNA	Deoxyribonucleic acid
DS	Danish standard
DSMZ	German Collection of Microorganisms and Cell Cultures
EN	European norm
EU	Endotoxin unit
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
FOF	Follow-on formula
FoodEx2	EFSA standardised food classification and description system
FS	Food supplements
FSMP	Food for special medical purposes
FSSC 22000	Food Safety System Certification 22000
Gal	D-Galactose
Glc	D-Glucose
GlcNAc	N-acetyl-D-glucosamine
GLP	Good Laboratory Practice
GMO	EFSA Panel on Genetically Modified Organisms
GMP	Good Manufacturing Practice
GOS	Galacto-oligosaccharides
GRAS	Generally Recognised As Safe
GRN	GRAS Notice
HACCP	Hazard Analysis Critical Control Points
HMBC	Heteronuclear multiple-bond correlation
HiMO	Human-identical milk oligosaccharide
HMO	Human milk oligosaccharide
HPAEC-PAD	High-performance anion-exchange chromatography – pulsed amperometric detection
HPLC-ESI	High-performance liquid chromatography – electrospray ionisation
HSQC	Heteronuclear single quantum correlation
IAC-HPLC-FD	Immunoaffinity chromatography – high-performance liquid chromatography – fluorescence detection
ICP-MS	Inductively coupled plasma – mass spectrometry
IF	Infant formula
ISO	International Organisation for Standardisation
IUPAC	International Union of Pure and Applied Chemistry
LFGB	German Food and Feed Code
LNT	Lacto-N-tetraose
LNnT	Lacto-N-neotetraose
LOQ	Limit of quantification
MRM	Multiple reaction monitoring
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
NANA	N-acetyl-D-neuraminic acid
ND	Not detected
NDA	EFSA Panel on Nutrition, Novel Foods and Food Allergens
NF	Novel food
NMR	Nuclear magnetic resonance spectroscopy
OECD	Organisation for Economic Co-operation and Development
Ph. Eur.	European Pharmacopeia
qPCR	Quantitative polymerase chain reaction
RH	Relative humidity
RNA	Ribonucleic acid
SD rats	Sprague Dawley rats
TG	Test guidelines
TS	Technical specification
US	United States
US FDA	US Food and Drug Administration
w/w	weight per weight

Annex A – Dietary exposure estimates to the Novel Food for each population group from each EU dietary survey

Information provided in this Annex is shown in an Excel file (downloadable at https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.7329#support-information-section).