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Safety of 3-FL (3-Fucosyllactose) as a novel food pursuant to Regulation (EU) 2015/2283

EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA), Dominique Turck, Jacqueline Castenmiller, Stefaan De Henauw, Karen Ildico Hirsch-Ernst, John Kearney, 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 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 also contains D-lactose and its monomers, L-fucose and a small fraction of other related saccharides. The NF is produced by fermentation with a genetically modified strain of *Escherichia coli* K-12. 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 in a variety of foods, including infant and follow-on formula, foods for infants and toddlers, foods for special medical purposes and food supplements. The target population is the general population, except for food supplements for which the target population is individuals above 1 year of age. The anticipated daily intake of 3-FL from the NF at the maximum proposed use levels is unlikely to exceed the intake level of breastfed 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. In infants below 1 year of age, a possible exceedance of a natural intake was observed, but the degree of this exceedance is not considered of safety concern in view of the wide range of 3-FL concentrations in human milk. Food supplements are not intended to be used if other foods with the added NF (as well as human milk for young children) 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-2019-00666

Correspondence: nda@efsa.europa.eu

Panel members: Dominique Turck, Jacqueline Castenmiller, Stefaan De Henauw, Karen Ildico Hirsch-Ernst, John Kearney, 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.

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1. Introduction

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

On 1 October 2019, the company DuPont Nutrition & Biosciences ApS submitted a request to the Commission in accordance with Article 10 of Regulation (EU) No 2015/2283[1] to place on the EU market 3-fucosyllactose as a novel food.

3-fucosyllactose is intended to be used in a number of foods and in food supplements as defined in Directive 2002/46/EC excluding food supplements for infants.

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-fucosyllactose as a novel food.

2. Data and methodologies

2.1. Data

The safety assessment of this novel food (NF) is based on data supplied in the application and information submitted by the applicant following EFSA requests for supplementary information.

During the assessment, the Panel identified additional data which were not included in the application: Albrecht et al. (2014), Brand-Miller et al. (1995, 1998), Choi et al. (2015), Erney et al. (2001), Gidrewicz and Fenton (2014), Gnoth et al. (2001), Gorbach (1978), Muhldorfer and Hacker (1994), Rijnierse et al. (2011), Rudloff and Kunz (2012), Samuel et al. (2019), Urashima et al. (2018).

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[2].

A common and structured format on the presentation of NF applications is described in the EFSA guidance on the preparation and presentation of an 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 (including both data 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:

- Strain Risk Assessment of the GMM (Annex 1 to the dossier)
- Manufacturing process of the Novel Food (Annex 2 to the dossier)
- Chemical analysis of five representative batches of the 3-FL Novel Food product (Annex 3 to the dossier)
- Chemical equivalence of 3-FL from different sources (Annex 4 to the dossier)
- Stability studies and Validation of detection methods for 3-FL (Annex 5 to the dossier)
- Intake assessment (Annex 7 to the dossier)
- Pharmacokinetic Study (ADME) (Annex 8 to the dossier): Analysis of 3-Fucosyllactose in Serum and Urine from the Subchronic Toxicity 90-Day Feeding Study in Rats
- Genotoxicity studies (Annex 10 to the dossier): Bacterial Reverse Mutation Test; In Vitro Mammalian Cell Micronucleus Test in Chinese Hamster Ovary Cells; Mouse Micronucleus Test; Chromosome Aberration Test in Human Lymphocytes in vitro
- Toxicity studies (Annex 11 to the dossier): Acute Oral Toxicity Study in Rats; Subchronic Toxicity 90-Day Feeding Study in Rats; 6-day Study in Neonatal Piglets; 3-Week Study in Neonatal Piglets.

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

[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) N0 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001 (2013/0435 (COD). OJ L 327, 11.12.2015, p. 1–22.

[2] 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, pp. 64–71.

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.

This assessment concerns only the risks that might be associated with the 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.

3. Assessment

3.1. Introduction

The NF's primary constituent is 3-fucosyllactose (3-FL), a trisaccharide consisting of L-fucose, D-galactose and D-glucose. 3-FL is naturally occurring in mammalian milk with the highest concentrations occurring in human milk and is therefore typically referred to as one of several human milk oligosaccharides (HMO). The NF is produced through fermentation by a genetically modified microorganism (GMM), and after subsequent purification, it consists of ≥ 90% of 3-FL, that is structurally identical to 3-FL occurring in human milk. The remaining ≤ 10% of the product is mainly composed of monosaccharides and di-saccharides, namely, L-fucose, D-glucose, D-galactose and D-lactose and a small fraction (≤ 3%) of other carbohydrates. The NF is proposed to be used in foods for infants and young children (including the use as human identical milk oligosaccharides (HiMO) in infant formulae (IF) and follow-on formulae), foods for special medical purposes, total diet replacements for weight control, food supplements, beverages and in a variety of other foods (e.g. dairy products, cereals). The target population is the general population.

According to Regulation (EU) 2015/2283, this NF falls under the following category:

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, which is a natural constituent of human milk. It also contains D-lactose and its monomers (glucose and galactose), L-fucose and a small fraction of other related saccharides resulting in a well-characterised mixture of carbohydrates (> 97.6% in representative batches, see Table 1). It is produced by fermentation with a genetically modified strain of *Escherichia coli* K12 MG1655. The main component is 3-FL, a trisaccharide comprised of D-galactose and D-glucose to which L-fucose is linked through an α-(1-3) bond: β-D-Gal-(1-4)-[α-L-Fuc(1-3)]-D-Glc. Synonym: α-L-Fuc-(1→3)-[β-D-Gal-(1→4)]-D-Glc. It is characterised by the molecular formula $C_{18}H_{32}O_{15}$. The molecular mass is 488.44 Da, CAS 41312-47-4 and IUPAC name β-D-galactopyranosyl-(1→4)[-α-L-fucopyranosyl-(1→3)]-D-glucopyranose. 3-FL is one of the most prevalent neutral milk oligosaccharides found in human milk (Erney et al., 2001; Thurl et al., 2017).

The structure of 3-FL has been confirmed by mono-dimensional and two-dimensional Nuclear Magnetic Resonance Spectroscopy (NMR), namely ^{1}H, ^{13}C HSQC (heteronuclear single quantum correlation) and ^{1}H, ^{13}C HMBC (heteronuclear multiple bond correlation) spectra. The ^{1}H and ^{13}C chemical shifts were assigned in agreement with the relevant literature (Kjærulff and Gotfredsen, 2014; Van Leeuwen et al., 2014). In addition, in the HMBC spectrum, the α-(1-3) linkage between fucose and glucose was unequivocally identified by long range ^{1}H and ^{13}C NMR correlations.

All major well-resolved signals in the NMR spectra of 3-FL are identical to those of a commercially available specimen and to the reference material derived from human milk demonstrating their equivalence (Figure 1).

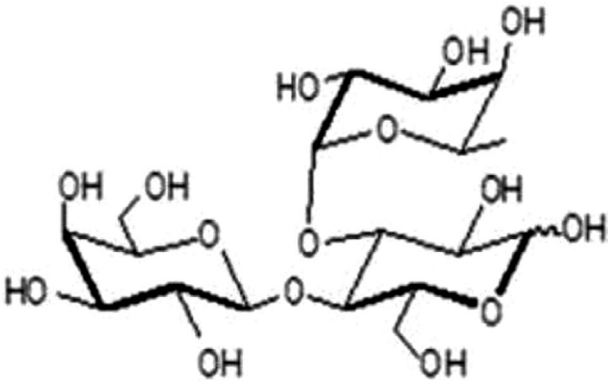

Figure 1: Structure of 3-FL

The 3-FL produced by the microbial fermentation described has been shown to be chemically and structurally identical to its naturally occurring counterpart present in human milk oligosaccharides by mono- and two-dimensional NMR and is therefore considered as HiMO.

3.3. Production process

According to the information provided by the applicant, the NF is produced in line to Good Manufacturing Practice (GMP) and Hazard Analysis Critical Control Points (HACCP) principles.

The 3-FL is produced by fermentation of carbohydrates using a genetically modified *E. coli* which excretes the 3-FL extracellularly. The cells are removed and the product goes through a series of purification, concentration and isolation steps to obtain the NF as a dried, amorphous powdered mixture.

The genetically modified production strain *E. coli* K-12 MG1655 INB001084 is a derivative of the parental strain *E. coli* K-12 MG1655. The complete genome of *E. coli* K-12 MG1655 INB001084 has been sequenced and compared to the complete sequence of the parental strain (ASM584v2 - Genome - Assembly - NCBI) as provided by Blattner et al. (1997). Although the species *E. coli* was considered not suitable for qualified presumption of safety (QPS) status (EFSA BIOHAZ Panel, 2018), *E. coli* K-12, from which the parental and producing strains are derived, is considered as a safe and non-pathogenic or toxigenic microorganism widely used for biotechnological applications (Gorbach, 1978; OECD, 1986; Muhldorfer and Hacker, 1994; U.S. EPA, 1997).

The production strain was constructed by engineering the parental strain via disruptions in genes that could interfere with the required metabolic pathway to effectively synthesise 3-FL. Next, four genes consisting of coding sequences plus artificial promoters and terminators were inserted in the genome or expressed from plasmid. A detailed description of the genetic modification steps applied to obtain the production strain has been provided by the applicant. The production strain has been deposited at BCCM/LMG Collection in Belgium.

The batch fermentation medium is based on sugars (lactose and sucrose), amino acid, salts and vitamins and is sterilised prior to use. The product formation and process progress are followed by monitoring of key substrate components as well as 3-FL product formation. The downstream purification includes several steps to ensure the purity of the product.

The initial post fermentation processing involves removing the cell biomass, endotoxins and large molecules e.g. proteins from the fermentation broth; this is achieved using common cell removal technologies such as nanofiltration. The cell-free permeate is then purified by using active carbon treatment, lactase hydrolysis of residual lactose and ion removal technologies such as ion exchange (IEX) membrane filtration to ensure a high-quality 3-FL product. Small molecules (e.g. organic acids, inorganic salts) formed/used in the fermentation stage and off-colour compounds are removed in the purification process. Potential microbial contaminants are then removed via sterile filtration and the resulting output is concentrated by vacuum evaporation to remove excess water. The final product is spray dried.

Most of the purification steps are done at low temperatures to prevent microbiological growth and ensure chemical stability of 3-FL. Some purification steps, such as evaporation, require higher temperature for optimal performance and product quality.

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

3.4. Compositional data

The NF contains 3-FL as primary ingredient (around 93% w/w dry matter). The remainder is a mixture of substances such as D-lactose, L-fucose, D-galactose, D-glucose and other carbohydrates structurally related to 3-FL (3-FL isomers, difucosyllactose isomer and oligomers based on combinations of deoxy-pentitols, hexitols and hexoses).

The Panel therefore notes that although the main component of the NF is 3-FL, other fractions (lactose and its monomers, fucose) are present in small amounts in the NF. Lactose is the most prevalent molecule in human milk (~ 7 g/100 mL) and its monomers glucose and 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). In representative batches of the NF, the average amount of lactose was around 2%, while the content of each of the other saccharides was around or below 1%.

Regarding the physico-chemical properties, the NF can be described as a white to off-white amorphous powder. It is readily soluble in aqueous solutions (up to 500 mg/mL at room 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 independent batches of the NF (Table 1). 3-FL and other carbohydrates have been analysed by HPLC-HILIC/RI (high-performance liquid chromatography-hydrophilic interaction liquid chromatography/refraction index) using in house validated methods as well as analyses by certified external laboratories. Small amounts of other carbohydrates were identified by HPLC-HILIC/MS (mass spectrometry). The applicant also provided the analyses of heavy metals, mycotoxins and microbial counts. The microbiological purity of batches of the NF has been assessed for non-pathogenic microorganisms (bacteria, yeasts and moulds) as general hygiene indicators, as well as for selected food-borne pathogens.

In three batches, the applicant also analysed the content of amino acids, biogenic amines, organic acid profile and microelements (data not included in the table): none of the tested amino acids, biogenic amines and organic acids were detected.

Table 1: Batch to batch analysis of the NF

Parameter (unit)	Batch A	Batch B	Batch C	Batch D	Batch E	Analytical methods
CARBOHYDRATE CONTENT (w/w DM)						
3-Fucosyllactose	91.5%	90.8%	93.4%	94.1%	93.2%	HPLC-HILIC/RI internal method
D-Lactose	2.2%	2.9%	0.2%	1.1%	2.3%	HPLC-HILIC/RI internal method
L-Fucose	1.1%	0.8%	1.9%	0.3%	0.3%	HPLC-HILIC/RI internal method
D-Galactose/ D-Glucose	1.2%	1.0%	2.0%	0.5%	0.4%	HPLC-HILIC/RI internal method
Other carbohydrates[(a)]	2.0%	2.7%	1.2%	1.6%	1.5%	HPLC-HILIC-MS/MS internal method
Total carbohydrates	98.0%	98.2%	98.7%	97.6%	97.7%	
CHEMICAL ANALYSIS						
Water content	3.4%	3.5%	3.7%	3.2%	3.0%	Karl-Fischer titration ISO 8534:2017
pH (5% solution)	6.0	6.1	5.5	6.3	5.5	Potentiometric pH measurement
Protein content (mg/kg)	≤ 25	≤ 25	≤ 25	≤ 25	≤ 25	Nanoquant (modified Bradford)
Total ash (%)	< 0.12	< 0.12	< 0.12	< 0.12	< 0.12	NMKL 173:2005, mod
Arsenic (mg/kg)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	DIN EN 15763:2010
Cadmium (mg/kg)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	DIN EN 15763:2010

Parameter (unit)	Batch A	Batch B	Batch C	Batch D	Batch E	Analytical methods
Lead (mg/kg)	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	DIN EN 15763:2010
Mercury (mg/kg)	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	DIN EN 15763:2010
Aflatoxin M1 (µg/kg)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	EN ISO 14501
Aflatoxin B1 (µg/kg)	< 1	< 1	< 0.1[(c)]	< 1	< 1	DIN EN 14123 mod
Endotoxins (EU/1,000 mg)	16	30	14	157	8	Ph. Eur. 2.6.14 + Interference study
GMO detection[(b)] (ng DNA/g)	Not detected	Not detected	Not detected	Not detected	Not detected	Internal protocol
MICROBIOLOGY ANALYSIS						
Standard Plate Count (CFU/g)	190	< 10	< 10	< 10	< 10	ISO 4833-1
Yeasts (CFU/g)	< 10	< 10	< 10	< 10	< 10	NMKL 98
Moulds (CFU/g)	< 10	< 10	< 10	< 10	< 10	NMKL 98
Enterobacteriaceae	Not detected in 10 g	Not detected in 10 g	Not detected in 10 g	Not detected in 10 g	Not detected in 10 g	ISO 21528-1
***Salmonella* spp.**	Not detected in 100 g	Not detected in 100 g	Not detected in 100 g	Not detected in 100 g	Not detected in 100 g	NMKL 71
Cronobacter (Enterobacter) sakazakii	Not detected in 100 g	Not detected in 100 g	Not detected in 100 g	Not detected in 100 g	Not detected in 100 g	ISO/TS 22964
Listeria monocytogenes	Not detected in 25 g	Not detected in 25 g	Not detected in 25 g	Not detected in 25 g	Not detected in 25 g	ISO 11290-1/Rapid L mono
***Bacillus cereus* (CFU/g)**	< 10	< 10	< 10	< 10	< 10	ISO 7932/NMKL 67

DM: dry matter; EU: endotoxin units; CFU: colony forming units; GMM: genetically modified microorganisms; GMO: genetically modified organism; LoD: limit of detection; LoQ: limit of quantification.
(a): 3-FL isomer, difucosyllactose isomer and carbohydrate oligomers.
(b): The GMO detection means absence of DNA from the GMM strain (LoD < 10 ng DNA/g of NF).
(c): For this batch a different sample matrix category characterised by LoQ of < 0.1 ug/kg has been used.

Since variability in endotoxin and standard plate counts was noted, the applicant was requested to provide additional data. Four additional batches showed that endotoxins were below 8 IU/g and Standard Plate Count below 40 CFU/g.

No residual DNA from the production strain was detected in five independent batches of the NF using quantitative polymerase chain reaction (qPCR) amplification of two inserted genes in the modified strain.

Information was provided on the accreditation of the laboratories that conducted the analyses presented in the application.

The Panel considers that the information provided on the composition of the NF is sufficient for characterising the NF.

3.4.1. Stability

Stability of the NF

The applicant performed stability tests with the five independently produced batches of the NF included in Table 1. The tests were carried out at normal storage conditions in commercial packaging at 25°C and at 60% RH for up to 24 months. The batches were analysed for the content of 3-FL, lactose, galactose + glucose, fucose, other carbohydrates and moisture. Microbiological analyses were conducted at time 0, in the middle and at the end of the stability test period (24 months).

The content of 3-FL was around 100% of original content in all five batches throughout the experiment. Accelerated stability at 40°C and at 75% RH over a 6-month period for the same five batches was also conducted. None of the measured parameters (levels of carbohydrates, moisture and microbiological parameters) were affected during storage.

Based on the data available, the Panel considers that the NF is expected to be stable for 24 months when stored at room temperature.

Stability of NF under the intended conditions of use

The applicant also tested the content of 3-FL during storage in various food matrices (whole milk, UHT milk, yoghurt, infant formula and cereal bars). In the whole milk, a stable content (0.97–1.1 mg/mL) over 22 days was recorded. In UHT milk, the content of 3-FL was stable over 91 days (from the first measured value of 0.87 on Day 7 to 0.84 mg/mL on Day 91). The applicant argues that the small drop at the first time point (vis-à-vis the calculated 0.95 mg/mL) could be a result of UHT treatment (142°C for 3 seconds) since 3-FL has been added prior to UHT treatment into the product. In yoghurt only 72% of the original added amount of NF was found after 35 days, and already on day 1 a decrease of about 25% was recorded. The applicant notes that 3-FL has been added prior to the pasteurisation process (95°C for 6 min) and thus the decrease of the content of 3-FL could be the result of either pasteurisation or fermentation with starter cultures at a low pH (4.6). During the following 34 days, the concentration of 3-FL was stable (Støvlbæk Christensen et al., 2020).

After 4 months at ambient temperature, 3-FL content was around 100% of the original in cereal bars. Similar results (94–100% of the original content) were also recorded after 6 weeks at accelerated storage condition (32°C).

The Panel considers 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.

3.5. Specifications

The specifications of the NF are indicated in Table 2.

Table 2: Specifications of the NF

Description: 3-fucosyllactose (3-FL) is a white to off-white powder that is produced by a microbial process	
Source: genetically modified strain of *E. coli* K-12	
Parameters	Specification
3-Fucosyllactose (w/w % DM)	≥ 90
D-Lactose (w/w % DM)	≤ 5
L-Fucose (w/w % DM)	≤ 3
D-Galactose/D-Glucose (w/w % DM)	≤ 3
Other carbohydrates[(a)] **(w/w % DM)**	≤ 3
Water (%)	≤ 5.0
pH (5% solution)	3.0–7.5
Protein (w/w %)	≤ 0.01%
Total Ash (%)	≤ 0.5
Arsenic (mg/kg)	≤ 0.2
Cadmium (mg/kg)	≤ 0.05
Lead (mg/kg)	≤ 0.05
Mercury (mg/kg)	≤ 0.1
Aflatoxin M1 (µg/kg)	≤ 0.025
Aflatoxin B1 (µg/kg)	≤ 0.1
Endotoxins (EU/g)	≤ 300
Microbiological parameters	
Standard Plate Count (CFU/g)	≤ 1,000
Yeasts and moulds (CFU/g)	≤ 100
Enterobacteriaceae	Not detected in 10 g
***Salmonella* spp.**	Not detected in 25 g

Cronobacter (Enterobacter) sakazakii	Not detected in 10 g
Listeria monocytogenes	Not detected in 25 g
***Bacillus cereus* (CFU/g)**	≤ 10

DM: dry matter; EU: endotoxin units; CFU: colony forming units.
(a): Other carbohydrates refers to 3-FL isomer, difucosyllactose isomer and oligomers.

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

The NF has no history of use.

Considering that 3-FL is a naturally occurring trisaccharide that is present in relevant amounts only in human milk, the history of human exposure to 3-FL is limited primarily to that of breastfed infants.

Although the HMO pattern depends on the genetic background of the mother and unlike some other HMOs (e.g. 2′–fucosyllactose (2′-FL) or difucosyllactose (DFL)), 3-FL is found in the human milk of most women (> 96%) in 10 countries studied (Erney et al., 2000, 2001). The Panel also noted that 3-FL is among the most represented HMOs and shows levels that are increasing over the course of the lactation. 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). Therefore, the intake of 3-FL from consumption of cow milk may be considered as very low.

3.6.2. Consumption of oligosaccharides constituent of the NF in human milk

Human milk contains a family of structurally related oligosaccharides, known as HMOs, as the third largest 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 amount and composition vary among 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 HMO and 3-FL show a wide variability of concentrations in human milk. From the recent systematic review by Thurl et al. (2017), the reported range of mean concentrations of 3-FL in human milk for lactating women is 0.24–1.24 g/L for 0–100 days lactation while the 95th percentile is 0.34–1.44 g/L, and concentrations are increasing over time. In another paper (Erney et al., 2001), an average of 1.39 and a maximum of 3.92 g/L for 3-FL have been reported. In a recent publication with European data, variability and concentrations increasing over the course of lactation have been confirmed, with averages ranging from 0.4 to 1.2 g/L and a maximum 75th percentile of 1.64 g/L (Samuel et al., 2019).

Based on the mean and the highest reported occurrence concentrations of 3-FL in human milk as reported by Thurl et al. (2017), and considering the average and high daily intake 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 3). 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. 3-FL concentrations from a similar lactation stage (after day 100) have been considered.

Table 3: Estimated daily intake levels of 3-FL from human milk (800 and 1,200 mL) for infants of 6.7 kg bw, based on mean and high concentration of 1.24 g/L and 1.44 g/L, respectively, of 3-FL in human milk from lactation day 100 onwards (Thurl et al., 2017)

	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	148	172	222	258

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, except for food supplements for which the target population is individuals above 1 year of age.

3.7.2. Proposed uses and use levels

The NF is proposed to be used as an ingredient in various food categories. These food products, defined using the FoodEx2 hierarchy, and the proposed maximum use levels are reported in Table 4.

The applicant also intends to market the NF as food supplement as defined in Directive 2002/46/EC for individuals above 1 year of age. Specifically, the maximum daily intake of 5 g, for individuals above 3 years of age or the maximum daily intake of 1.2 g for children aged 1–3 years are proposed.

For foods for special medical purposes, the applicant did not propose maximum use levels and the Panel considers that the maximum use levels of the NF should not be higher than the maximum levels specified for the proposed food uses or the maximum daily intake proposed for food supplements.

Food supplements are not intended to be used if other foods with added NF or human milk are consumed on the same day.

Table 4: FoodEx2 categories and maximum use levels of the NF used in the refined estimate of the anticipated daily intake of the NF using individual data from EU dietary surveys

FoodEx2 code	FoodEx2 level	Food category	Maximum use level of the NF mg/100 g
A02LV	5	Cow milk	85
A0CXA	5	European buffalo milk	85
A02MC	5	Sheep milk	85
A02MB	4	Goat milk	85
A02MV	3	Butter milk	85
A02NQ	4	Yoghurt drinks	50
A02NR	4	Probiotic milk-like drinks	50
A02NV	5	Kefir	50
A02NE	4	Yoghurt	500
A03TH	4	Milk imitates	85
A03TV	5	Soy yoghurt	850
A03TZ	5	Imitation yoghurt, non-soy	850
A00EY	4	Cereal bars	3,000
A00EZ	4	Cereal bars plain	3,000
A00FA	4	Cereal bars mixed	3,000
A03PZ	4	Infant formulae, powder	680
A03QE	4	Infant formulae, liquid	85
A03QK	4	Follow-on formulae, powder	680
A03QQ	4	Follow-on formulae, liquid	85
A03QY	3	Simple cereals which have to be reconstituted	2,100
A0BZE	3	Simple cereals for infants and children reconstituted	300
A03QZ	3	Cereals with an added high protein food which have to be reconstituted	1,200
A0BZF	3	Cereals with added high protein food reconstituted	300
A03RA	3	Biscuits, rusks and cookies for children	300
A03RC	2	Ready-to-eat meal for infants and young children	300
A03RB	3	Pasta for children	300
A03RP	3	Special food for children's growth	300
A03RN	3	Fruit and vegetable juices and nectars specific for infants and young children	85

FoodEx2 code	FoodEx2 level	Food category	Maximum use level of the NF mg/100 g
A03RT	4	Total daily diet replacement for weight reduction	3,000
A0EQN	5	Soft drinks with minor amounts of fruits or flavours	100
A03EA	5	Soft drink with fruit juice (fruit content below the minimum for nectars)	100
A03EX	5	Soft-drink, flavoured, no fruit	100
A03GA	4	Energy drink	100
A03GB	4	Isotonic and sport drinks	100

3.7.3. Anticipated intake of the NF

Anticipated intake of the NF from the consumption of infant formula 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 (0.85 g/L in IF), the high intake of the NF from IF alone is estimated for an infant of 6.7 kg to be 221 mg/kg bw per day.

The Panel notes that the anticipated daily intake of the NF from the consumption of IF (only) does not exceed the estimated high daily intake of 3-FL (i.e. 258) in breastfed infants per kg/bw (Table 3).

Anticipated intake of the NF from the proposed uses and use levels of the NF

EFSA performed an intake assessment of the anticipated daily intake of the NF based on the applicant's proposed uses and maximum proposed use levels (Table 4), using individual data from the EFSA Comprehensive European Food Consumption Database (EFSA, 2011). The lowest and highest mean and 95th percentile anticipated daily intake of the NF (on a mg/kg body weight (bw) basis), among the EU dietary surveys, are presented in Table 5.

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 5: Intake estimate resulting from the use of the NF 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)		P95 intake (mg/kg bw per day)	
		Lowest[(a)]	Highest[(a)]	Lowest[(b)]	Highest[(b)]
Infants	< 1	33	138	83	361
Young children[(d)]	1–< 3	28	100	80	247
Other children	3–< 10	12	49	31	119
Adolescents	10–< 18	4	16	16	38
Adults[(c)]	≥ 18	6	10	20	24

(a): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 22/4/2021. The lowest and the highest averages observed among all EU surveys are reported in these columns.
(b): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 22/4/2021. 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): Includes elderly, very elderly, pregnant and lactating women.
(d): Referred as 'toddlers' in the EFSA food consumption comprehensive database (EFSA, 2011).

The Panel notes that the content of 3-FL in the NF accounts for about 93%, therefore the figures that are calculated considering a 100% purity slightly overestimate the actual intake. The Panel also notes that the highest 95th percentile (i.e. 361 mg/kg bw, recorded in only one out of 13 dietary surveys included in the EFSA food consumption database) exceeds the high estimate for 3-FL from human milk (i.e. 258 mg/kg bw) in infants (up to and including 11 months).

3.7.4. Anticipated use as a food supplement

The applicant has proposed a maximum daily intake of 5 g of the NF as food supplements for individuals above 3 years of age or at a maximum level of 1.2 g/day for young children (12–35 months).

Table 6: Use of the NF as food supplement and resulting intake expressed as mg/kg bw per day

Population group	Age (years)	Body weight[(a)] (kg)	Use level (g/day)	Intake (mg/kg bw per day)[(b)]
Infants	< 1	5	–	–
Young children	1–< 3	12	1.2	100
Other children	3–< 10	23.1	5	216
Young adolescents	10–< 14	43.4	5	115
Old adolescents	14–< 18	61.3	5	82
Adults	≥ 18	70	5	71

(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' are calculated by considering the use levels in 'mg/d' and default body weights defined in EFSA Scientific Committee, 2012.

The maximum daily intake from food supplements of the NF (i.e. 5 g/day) results in a maximum daily intake ranging from 71 (adults) to 216 (other children) mg/kg bw in the general population (Table 6). The maximum dose of 1.2 g/day in young children (body weight of 12 kg) results in a maximum intake of 100 mg/kg bw (default body weight values from EFSA Scientific Committee (2012)).

The Panel notes that the maximum daily intake of 3-FL from the use of NF as food supplement (i.e. from 1.2 to 5 g/day) does not exceed the estimated high daily intake of 3-FL from human milk calculated for infants on a body weight basis (Table 3) for any population category.

Food supplements are not intended to be used if other foods with added 3-FL are consumed on the same day. For young children, food supplements are not intended to be used if human milk or other foods with added NF are consumed on the same day.

3.7.5. Combined intake from the NF and other sources

The Panel notes that the NF is not authorised for use in food categories other than those proposed for the NF under assessment. Therefore, the only relevant additional source for these oligosaccharides is human milk.

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

HMOs, including fucosyllactoses, are considered 'non-digestible oligosaccharides' (EFSA NDA Panel, 2014) 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).

The applicant has conducted experimental activities to assess systemic availability of 3-FL. In the *in vivo* micronucleus study conducted in mice with the NF (see section 3.11.1; study report 2018c), four additional mice/sex were treated by oral gavage at the dose of 500 mg 3-FL/kg bw and were sampled to assess plasma levels at 4 h after dosing. This was to demonstrate the possible target cells exposure to 3-FL after oral administration. The plasma analysis revealed levels of 600–700 ng/mL confirming some degree of absorption and the systemic availability of 3-FL.

In the 90-day rat study where the NF was mixed into the diet at concentrations of 5 or 10% (see Section 3.11.3; Unpublished study report, 2019c,d), blood and urine samples were collected from all rats of the main study on study day 80 or 81. Animals were housed overnight in metabolic cages with access to feed and water to collect urine over at least 16 h. Approximately at 6 and 10 a.m. and 2 p.m., three to four animals/sex per group for each time point were sampled to obtain serum for 3-FL levels determination. These activities have been conducted according to GLP (Good Laboratory Practices) and experimental design, results and analytical procedures are included in a separate technical report (Pitt et al., 2019; study report, 2019c,d). The doses calculated on the day of sampling correspond to approximately 2,300 and 4,700 mg/kg bw per day in males and 3,200 and 5,900 mg/kg bw per day in females, for 5 and 10% diet, respectively. The systemic exposure of 3-FL was

determined by analysis at the three time points over an 8-h period. The pharmacokinetic evaluation was reported to be indicative of a low absorption (< 1%) of the daily dietary intake and the limited systemic exposure from dietary intake showed dose proportionality. Exposure was higher in female rats than in males and urinary excretion was ~ 0.4% of the administered dose in both dose groups regardless of sex.

In the literature, it is reported that in humans, a fraction of low molecular weight HMOs, including 3-FL, are absorbed intact into the circulatory system and excreted in the urine without metabolic modification. Levels found are in correlation with their dietary intake from human milk. The relative fractions of HMOs were low, 0.1% of milk levels for plasma and 4% of milk levels for urine (Goehring et al., 2014). 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).

In *in vivo* ^{13}C-labelling studies, it was shown that when lactating women received an oral bolus of ^{13}C-labelled galactose, their mammary glands incorporated the label during HMO synthesis (Obermeier et al., 1999; Rudloff et al., 2006, 2012). The breastfed infant then ingested the *in vivo*-labelled HMO with the mother's milk and in the infant's urine < 1% of labelled HMO was found.

Based on information available on HMOs and experimental data provided, the Panel considers that only small amounts are expected to be absorbed intact in the upper GI tract and then excreted in the urine.

Moreover, the Panel assumes that the absorption and metabolic fate of 3-FL or other components of the NF do not differ from that of similar components found in human milk.

3.9. Nutritional information

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

The Panel considers that consumption of the NF at the proposed use levels is not nutritionally disadvantageous.

3.10. Toxicological information

The applicant provided eight toxicological studies on the NF, which were conducted in compliance with Organisation for Economic Co-operation and Development (OECD) principles of Good Laboratory Practice (GLP) (OECD, 1998) and in accordance with the relevant test guidelines (TG) from the OECD (with the exception of piglet studies). The studies were conducted with a unique NF batch (a blend of three different batches), which contained 94.6% of 3-FL. The applicant also provided a review paper (Pitt et al., 2019) where results from all the toxicity studies are reported, except the studies on piglets. The studies which were claimed proprietary by the applicant are listed in Table 7.

Table 7: List of toxicological studies with the NF provided by the applicant

Reference	Type of study	Test system	Dose
Unpublished study report (2018a), Pitt et al. (2019)	Bacterial reverse mutation test (OECD TG471)	*S. typhimurium* TA98, TA100, TA1535 and TA1537; *E. coli* WP2 uvrA	Up to 5,000 μg/plate (absence and presence of S9 mix)
Unpublished study report (2018b), Pitt et al. (2019)	*In vitro* mammalian cell micronucleus Test (OECD TG487)	Chinese hamster ovary cells	500; 1,000; 2,500; 3,500 and 5,000 μg/mL (absence and presence of S9 mix)
Unpublished study report (2019a), Pitt et al. (2019)	Chromosome aberration test *in vitro* (OECD TG473)	Cultured human lymphocytes	1,250; 2,500 and 5,000 μg/mL (absence and presence of S9 mix)
Unpublished study report (2018c), Pitt et al. (2019)	*In vivo* micronucleus Test (OECD TG474)	Crl:CD(ICR) mice	500; 1,000; 2,000 mg/kg bw
Unpublished study report (2018d), Pitt et al. (2019)	GLP Acute oral toxicity study	Crl:CD(SD) Female rats	5,000 mg/kg bw

Reference	Type of study	Test system	Dose
Unpublished study report (2018e), Pitt et al. (2019)	90-day GLP feeding study (OECD TG408)	Crl:CD(SD) M and F rats	5% and 10% of diet
Unpublished study report (2019b)	6-day oral study	Preweaning Landrace crossbred swine farm M and F piglets	1 and 2 g/L milk
Unpublished study report (2019c)	21-day GLP oral study	Preweaning Landrace crossbred swine farm M and F piglets	1 and 2 g/L milk

3.10.1. Genotoxicity

The potential genotoxicity of the NF was investigated in a bacterial reverse mutation test, an *in vitro* mammalian cell micronucleus test, an *in vitro* chromosomal aberration test in human lymphocytes and also in an *in vivo* micronucleus test in mice (Table 7).

The *in vitro* assessment of the mutagenic potential of the NF (unpublished study report, 2018a) was performed with histidine-dependent auxotrophic mutants of *Salmonella typhimurium*, strains TA1535, TA1537, TA98 and TA100, and a tryptophan-dependent mutant of *Escherichia coli*, strain WP2 uvrA (pKM101). A dose range preliminary test and a mutagenicity test were conducted with plate incorporation method at eight different concentrations from 333 up to 5,000 μg/plate (main study) either in the presence or absence of liver microsomal fractions (S9 fraction) with the NF in water solution. No substantial, reproducible or dose-related increases in revertant colony numbers over control counts were observed with any of the strains following exposure to 3-FL at any concentration. No appreciable toxicity or precipitation was observed following exposure to any dose of the NF. Therefore, the NF showed to be non-mutagenic at concentrations up to 5,000 μg/plate of 3-FL, in the absence or presence of metabolic activation.

In the *in vitro* mammalian cell micronucleus test in the CHO (Chinese hamster ovary - unpublished study report, 2018b) cells, concentrations of 3-FL from 500 up to 5,000 μg/mL (main test) were tested to assess the potential of 3-FL to cause an increase in the induction of micronuclei in *in vitro* cultured CHO cells after 4 or 24 h exposure in the presence or absence of metabolic activation (S9 fraction). No toxicity to cells or precipitation have been observed and the percentage of micronuclei was not significantly increased in any of the test substance concentrations. Although all values resulted to be within the 95% historical negative control limits, evidence of a statistically significant ($p \leq 0.05$, William's test) increasing concentration-related trend in 4 h with metabolic activation was noted. For this reason, a confirmatory assay with concentrations ranging from 500 to 5,000 μg/mL was conducted. In this additional test, similar results were obtained and substantial cell toxicity (55 ± 5% mitotic reduction) was observed at 1,000 and 5,000 μg/mL. Therefore, according to the relevant OECD TG (487, 2016), the results were considered as equivocal.

Finally, an *in vitro* human lymphocytes chromosomal test (unpublished study report, 2019a) was performed with the NF at concentrations ranging from 78 up to 5,000 μg/mL in the presence or absence of metabolic activation (S9 fraction) after 4 or 24 h of exposure. No dose-dependent toxicity to cells or precipitation has been observed and the NF did not induce any statistically significant increases in the frequency of structural and numerical aberrations in any of the three exposure conditions. The NF did not show any evidence of clastogenicity in the absence and presence of metabolic activation.

3-FL was also evaluated for its ability to induce micronuclei *in vivo* in bone marrow of Crl:CD1(ICR) mice (unpublished study report, 2018c) by analysing peripheral blood reticulocytes from five males and five females (seven males and seven females at the highest dose) mice 48 and 72 h after a single oral administration of 500, 1,000 and 2,000 mg/kg bw of 3-FL. Additionally, four mice per sex were also sampled to assess 3-FL plasma levels (pooled) at 4 h after dosing at 500 mg/kg. Plasma levels recorded demonstrated systemic exposure to 3-FL (see ADME Section 3.8). In addition, a statistically significant decrease in peripheral blood reticulocytes that is considered indicative of the target exposure in male mice (at the highest dose, with statistically significant decreasing dose-related trend) was also noted. No statistically significant or biologically relevant increases in the micronucleated reticulocyte frequency were observed in any group of mice treated with the NF.

Taking into account the test 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. Acute and subacute toxicity

The applicant has provided an acute toxicity study in rats. The Panel considers that in general acute toxicity studies are not pertinent for the safety assessment of NFs.

In the 6-day piglet studies (unpublished study report, 2019b), a batch of NF with a 3-FL content of 95% has been used. In this preliminary study, two male and two female piglets were given a dose of ~ 975 mg/kg bw per day (500 mL/kg bw per day of 2 g/L 3-FL in milk replacer offered via dish feeding). The author reported that no deaths or adverse effects on clinical observations, body weight, food consumption and food efficiency, clinical pathology or macroscopic observations in any of the animals on study were noted.

In the main 21-day GLP study (unpublished study report, 2019c, Appendix A), the same batch was used. Six male and female neonatal piglets were given the NF at 1 or 2 g/L of 3-FL in 500 mL/kg bw (in milk replacer six times a day) corresponding to an approximate average dose of 450 and 900 mg/kg bw per day at the two dose levels of 3-FL. In an additional group, fructo-oligosaccharide at 2 g/L was used as a comparator. No deaths or clinical signs were noted. A slight non-statistically significant reduction in food consumption and body weight was observed for piglets receiving the highest dose of 3-FL, mainly in males. A slight variation in some haematological parameters was recorded at 2 g 3-FL/L in males on Day 22, related to red blood cells (with statistical significance for the mean corpuscular volume and haemoglobin concentration). Small statistically significant decrease in alkaline phosphatase was noted with some time and dose correlation, mainly at the dose of 2 g/L for both 3-FL and fructo-oligosaccharide. In males at the highest 3-FL dose, a small decrease in globulins was also recorded. No other variations in chemical chemistry and coagulation parameters were noted. No gross pathology or histological alterations were noted. Although no clear test article-related findings were recorded and the administered doses appeared well tolerated, there were signs considered related to a reduced food intake at the highest dose of 3-FL in male piglets.

3.10.3. Subchronic toxicity

A 90-day GLP rodent feeding toxicity study according to OECD TG408 (1998) has been conducted with the NF.

Groups of 10 Crl:CD(SD) rats/sex were administered a powdered standard diet (control) or diet with 5% or 10% of NF starting from approximately 7 weeks of age for at least 90 days, until the day before necropsy (unpublished study report, 2018e). An additional control group was treated with fructo-oligosaccharide powder 10%, to compare any effects related to the general fibre-like characteristics at the same high dose. Blood and urine samples were collected from all rats of the main study during study week 12 for possible determination of plasma and urine levels of 3-FL (see Section 3.8). An average intake of 5,975 and 7,270 mg 3-FL/kg bw per day at 10% and 3,038 and 3,870 mg 3-FL/kg bw per day at 5% was calculated for male and female rats, respectively.

There were no deaths or effects of the NF on clinical observations (including ophthalmoscopic and neurobehavioural examination), clinical pathology investigation, macroscopic observations, organ weights or microscopic observations in this study. Only sporadic statistically significant variations in food consumption were noted through the study.

The authors considered that the no observed adverse effect level (NOAEL) of this study is the highest dose tested (corresponding to 5,975 and 7,270 mg 3-FL mg/kg bw per day as an average in male and female rats, respectively). The Panel agrees with this conclusion.

3.10.4. Human data

No human intervention studies with the NF have been provided by the applicant and no reference to human data was made.

3.11. Allergenicity

The protein content in the NF is low as indicated in the specifications (Table 2). The four proteins expressed in the *E. coli* strain were analysed using an algorithm, AllerTOP, predicting allergens (Dimitrov et al., 2013). None of the four proteins was found to be predicted as an allergen.

The Panel considers for these reasons that the NF is unlikely to trigger allergic reactions in the target population under the proposed conditions of use.

4. Discussion

The NF which is the subject of the application is a powdered mixture mainly composed of 3-FL, and also containing D-lactose and its monomers, L-fucose and a small fraction of other related saccharides, resulting in a well-characterised mixture of carbohydrates (> 97.6% in representative batches). The NF is obtained by microbial fermentation with a genetically modified strain of *E. coli* K-12.

The applicant intends to add the NF to a variety of foods (e.g. milk, yoghurt, cereals), including infant formula and follow-on formula, foods for infants and young children, foods for special medical purposes and food supplements. The target population is the general population, except for food supplements, for which the target population is individuals above 1 year of age.

In a 90-day dietary toxicity study conducted in rats fed with the NF at a concentration of 5 or 10% in the diet, the high dose (corresponding to ~ 6,000 and 7,000 mg 3-FL/kg bw in males and females, respectively) was identified as the NOAEL. When comparing the NOAEL from the 90-day toxicity study with the highest estimated exposure per population category (at 95th percentile; Table 5), the margins of exposure are relatively low, with range from about 17 to 250. The Panel notes that because of risk of nutritional imbalance with substances of this nature the maximum feasible doses that can be used in subchronic studies are only able to ensure a relatively low margin of exposure with respect to the highest estimated daily intakes in the intended population.

Considering that 3-FL is a naturally occurring oligosaccharide present in human milk and only very low concentrations of fucosylated oligosaccharides are found in bovine milk, the history of human exposure relates to breastfed infants. The Panel notes that other HiMOs (e.g. 2′-FL, LNT or 6′-SL) produced by fermentation from a similar genetically modified parental *E. coli* strain have been recently assessed (EFSA NDA Panel, 2019a,b, 2020a,b) and authorised for use as a NF.

The Panel also notes that the anticipated daily intake of 3-FL in the NF 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 body weight basis. In consideration of the wide variability observed in human milk levels and the conservative assumption underlying the estimated intake, the exceedance at high (95th percentile) intake noted in infants below 1 year of age (in only one out of 13 dietary surveys included in the EFSA food consumption database) does not raise safety concerns. The anticipated daily intake of the NF in the other population categories is unlikely to exceed the highest intake level of 3-FL in breastfed infants on a body weight basis. Since the intake in breastfed infants on a body weight basis is expected to be safe also for other population groups, the Panel considers that the intake of the NF for the proposed uses at their respective maximum use levels can be considered safe.

The maximum daily intake of 3-FL as a food supplement at the proposed maximum levels (i.e. from 1.2 g to 5 g/day) for the respective population categories also does not exceed the highest intake level of 3-FL in breastfed infants per kg bw. Food supplements are not intended to be used if other foods with added 3-FL (as well as human milk for young children) are consumed on the same day. The Panel notes, however, that for adolescents and adults, the concurrent intake as a food supplement does not exceed the natural intake by breastfed infants on a body weight basis.

It is finally noted that, in line with other oligosaccharides that are natural components of human milk, the safety assessment of this NF is mainly based on the comparison between the natural intake in breastfed infants and the estimated intake as NF. The same considerations apply for lactose and other mono- and oligosaccharides that are present as a very small fraction in the NF and that are natural milk components and considered of no safety concern.

5. Conclusions

The Panel concludes that the NF, composed of 3-FL and other structurally related mono- and oligosaccharides, is safe under the proposed conditions of use, including the use as a food supplement. The target population is the general population except for food supplements, for which the target population is individuals above 1 year of age.

Food supplements are not intended to be used if other foods with added 3-FL (as well as human milk for young children) are consumed on the same day.

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:

- Annexes to the dossier which relate to the Risk Assessment of the GMM, the identity, the manufacturing process, composition, stability and analytical methods (see Annexes indicated in Section 2.1)
- Genotoxicity studies (Annex 10 to the dossier): Bacterial Reverse Mutation Test; In Vitro Mammalian Cell Micronucleus Test in Chinese Hamster Ovary Cells; Mouse Micronucleus Test; Chromosome Aberration Test in Human Lymphocytes in vitro
- Pharmacokinetic Study (ADME) and Toxicity studies (Annex 8 and 11 to the dossier): Acute Oral Toxicity Study in Rats; Subchronic Toxicity 90-Day Feeding Study in Rats with serum and urine analysis; 6-day Study in Neonatal Piglets; 3-Week Study in Neonatal Piglets.

6. Recommendation

The Panel recommends that, given that several milk oligosaccharides have been authorised, the cumulative dose of this and other milk oligosaccharides from consumption of infant- and follow-on formula should not exceed the physiological levels from human milk.

7. Steps taken by EFSA

1) On 29/01/2020 EFSA received a letter from the European Commission with the request for a scientific opinion on the safety of 3-fucosyllactose as a novel food. Ref. Ares (2020)549014.
2) On 29/01/2020, a valid application on 3-fucosyllactose, which was submitted by Dupont Nutrition & Biosciences ApS, was made available to EFSA by the European Commission through the Commission e-submission portal (NF 2019/1321) and the scientific evaluation procedure was initiated.
3) On 09/06/2020, 16/11/2020 and 12/02/2021 EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4) On 21/09/2020, 16/01/2021 and 13/04/2021 additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
5) During its meeting on 25/05/2021, 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.

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Abbreviations

2′-FL	2′-Fucosyllactose
3-FL	3-Fucosyllactose
6′-SL	6′-Sialyllactose
ADME	absorption, distribution, metabolism and excretion
BCCM/LMG	Belgian Co-ordinated Collections of Micro-organisms (Laboratory of Microbiology, Ghent University)
BIOHAZ	Panel on Biological Hazards
bw	body weight
CAS	Chemical Abstract Services
CFU	colony forming units
CHO	Chinese hamster ovary
Da	Dalton
DFL	difucosyllactose
DIN	Deutsches Institut für Normung
DM	dry matter
DNA	Deoxyribonucleic Acid
EU	endotoxin units
Eur.Ph.	European Pharmacopoeia
FDA	US Food and Drug Administration
FEEDAP	Panel on Additives and Products or Substances used in Animal Feed
Gal	galactose
Glc	glucose
GlcNAc	N-acetyl-D-glucosamine
GLP	Good Laboratory Practice
GMM	Genetically Modified Microorganisms
GMO	Genetically Modified Organism
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis Critical Control Points
HiMO	human identical milk oligosaccharide
HMO	human milk oligosaccharide
HPLC-HILIC/RI	High Performance Liquid Chromatography-Hydrophilic Interaction Liquid Chromatography/Refraction Index
HMBC	heteronuclear multiple bond correlation
HSQC	heteronuclear single quantum coherence
IEX	ion exchange
IF	infant formula
ISO	International Organization for Standardization
IU	International Unit
IUPAC	International Union of Pure and Applied Chemistry
LNT	lacto-N-tetraose
LOD	limit of detection
LOQ	limit of quantification
MS	mass spectrometry
MS/MS	tandem mass spectrometry
NDA	Panel on Nutrition, Novel Foods and Food Allergens
NF	novel food
NMKL	Nordic Committee on Food Analysis
NMR	nuclear magnetic resonance spectroscopy
NOAEL	no observed adverse effect level
NMR	nuclear magnetic resonance spectroscopy
OECD	Organisation for Economic Co-operation and Development
QPCR	quantitative polymerase chain reaction
QPS	qualified presumption of safety
RH	relative humidity
TG	Test Guidelines

UHT	ultra-high temperature
US EPA	United States Environmental Protection Agency
w/w	weight for weight

Appendix A – Summary of the 3-week oral toxicity study in neonatal piglets

Study title	**GLP 3-week study on 3-FL in neonatal piglets** (Unpublished study report, 2019c)
Tested system	Domestic Landrace crossbred swine
Test material	The novel food, a unique batch (purity 95% of 3-FL)
Dose/concentration (route of administration)	Control group: 0 (standard milk replacer), 6 animals/sex Comparative control group: 2 g fructo-oligosaccharides (FOS)/L standard milk replacer, 6 animals/sex Dose group: 1 and 2 g 3-FL/L standard milk replacer, 6 animals/sex
Method	U.S. FDA Guidance for Industry: Nonclinical Safety Evaluation of Pediatric Drug Products, 2006

Key results

Parameters	**Sex**	**Dose groups (expressed in g/L reconstituted milk)**			
		0 (control, G1); mean ± SD	**2 g/L FOS (comparative control, G2); mean ± SD**	**1 g/L (low dose, G3); mean ± SD**	**2 g/L (high dose, G4); mean ± SD**
Haematology					
Mean corpuscular volume (MCV) fL (Day 22)	M	58.2 ± 1.6	58.0 ± 2.2	59.4 ± 1.8	54.5 ± 1.2*
	F	59.0 ± 2.1	58.5 ± 1.9	58.0 ± 3.0	58.9 ± 2.0
Haemoglobin (Hb) g/dL (Day 7)	M	10.4 ± 1.2	10.7 ± 0.5	10.6 ± 0.6	10.4 ± 0.9
	F	10.4 ± 1.5	10.4 ± 0.4	11.6 ± 1.0	11.6 ± 0.8**
Clinical chemistry					
Alkaline phosphatase (ALP) U/L (Day 7)	M	875 ± 242	769 ± 96	953 ± 201	695 ± 61
	F	1112 ± 213	869 ± 237	845 ± 149	930 ± 183
Alkaline phosphatase (ALP) U/L (Day 22)	M	818 ± 210	521 ± 116	788 ± 132	557 ± 97**
	F	802 ± 216	494 ± 58	653 ± 127	560 ± 109
Globulin g/dL (Day 22)	M	1.6 ± 0.1	1.7 ± 0.1	1.5 ± 0.1	1.5 ± 0.1.**
	F	1.7 ± 0.2	1.7 ± 0.2	1.6 ± 0.1	1.6 ± 0.2

Organ weight values					
Brain (g)	M	48.6 ± 1.8	50.1 ± 1.5	49.1 ± 1.7	46.7 ± 1.8**
	F	49.1 ± 2.5	49.4 ± 3.5	50.0 ± 3.1	49.3 ± 1.8
Large intestine, colon/bw (%)	M	0.52 ± 0.07	0.68 ± 0.06	0.67 ± 0.10	0.64 ± 0.06**
	F	0.12 ± 0.03	0.15 ± 0.05	0.16 ± 0.04	0.17 ± 0.05
Spleen (g)	M	17.2 ± 3.4	21.4 ± 5.7	22.2 ± 6.2	14.3 ± 5.7
	F	17.8 ± 1.9	22.0 ± 6.0	24.9 ± 4.7**	17.9 ± 3.4
Thyroid (g)	M	0.73 ± 0.14	0.96 ± 0.18	0.77 ± 0.22	0.66 ± 0.10***
	F	1.7 ± 0.2	1.7 ± 0.2	1.6 ± 0.1	1.6 ± 0.2

* ANOVA $p < 0.01$; ** ANOVA $p < 0.05$; *** $p < 0.05$ vs FOS.

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.6662#support-information-section).