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Safety of 2′-fucosyllactose (2′-FL) produced by a derivative strain (*Escherichia coli* SGR5) of *E. coli* W (ATCC 9637) as a Novel Food pursuant to Regulation (EU) 2015/2283

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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 2′-fucosyllactose (2′-FL) as a novel food (NF) pursuant to Regulation (EU) 2015/2283. The NF is mainly composed of the human-identical milk oligosaccharide (HiMO) 2′-FL, but it also contains D-lactose, L-fucose, fucosylgalactose, difucosyllactose, D-glucose and D-galactose, and a small fraction of other related saccharides. The NF is produced by fermentation by a genetically modified strain (*Escherichia coli* SGR5) of *E. coli* W (ATCC 9637). The information provided on the identity, manufacturing process, composition and specifications of the NF does not raise safety concerns. The applicant applies for the same use and use levels as already authorised for 2′-FL and included in the Union list of NFs, with the general population as target population. The Panel noted that the available intake estimate is not recent (2015) and based on a different database (2008–2010 UK data) than that used by EFSA. For this reason, the Panel decided to perform a new intake estimate according to the current EFSA approach. The Panel notes that the highest P95 daily intake of the NF from the use as food ingredient is higher than the estimated natural highest mean daily intake in breastfed infants and marginally higher in young children. The applicant also proposes to extend the use of 2′-FL in food supplements (FS) for infants at the use level of 1.2 g/day. The resulting estimated intake in infants from the proposed use in FS is within the natural intake of 2′-FL in breastfed infants. FS are not intended to be used if other foods with added 2′-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: 2′-fucosyllactose, 2′-FL, human milk oligosaccharide, HMO, HiMO, novel food, safety

Requestor: European Commission

Question number: EFSA-Q-2021-00407

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1. Introduction

1.1. Background and terms of reference as provided by the requestor

On 23 March 2021, the company Kyowa Hakko Bio Co., Ltd. submitted a request to the Commission in accordance with Article 10 of Regulation (EU) 2015/2283[1] to place on the EU market 2′-fucosyllactose (2′-FL) as a novel food (NF).

2′-FL is intended to be used in a number of food categories.

The applicant has requested data protection under Article 26 of Regulation (EU) 2015/2283 for data in support of this request.

In accordance with Article 10(3) of Regulation (EU) 2015/2283, the European Commission (EC) asks the European Food Safety Authority (EFSA) to provide a scientific opinion on 2′-FL as a NF.

In this opinion on 2′-FL, EFSA should also document whether and to what extent the requirements of Article 26(2)(c) of Regulation (EU) 2015/2283 are fulfilled regarding the data for which the applicant is requesting data protection.

1.2. Additional information

2′-FL is included in the Union list of authorised NFs (Commission Implementing Regulation (EU) 2017/2470[2]) when chemically synthesised (Commission Implementing Decision (EU) 2016/376[3]) (EFSA NDA Panel, 2015a,b) or produced by fermentation by genetically modified strains of *Escherichia coli* K-12 DH1 (Commission Implementing Regulation (EU) 2019/338[4]), *E. coli* BL21 (DE3) (Commission Implementing Regulation (EU) 2017/2201[5]) or *Corynebacterium glutamicum* ATCC 13032 (Commission Implementing Regulation (EU) 2023/859[6]) (EFSA NDA Panel, 2022a). Moreover, a 2′-FL/difucosyllactose (DFL) mixture produced by a genetically modified strain of *E. coli* K-12 DH1 (EFSA NDA Panel, 2019a), and 3-fucosyllactose (3-FL), a constitutional isomer of 2′-FL produced by genetically modified strains of *E. coli* K-12 MG1655 (EFSA NDA Panel, 2021) or *E. coli* BL21 (DE3) (EFSA NDA Panel, 2022b), are also included in the Union list of authorised NFs. The extension of use in food supplements (FS) for infants of 2′-FL and the 2′-FL/DFL mixture, both produced by genetically modified strains of *E. coli* K-12 DH1, and the safety of 3-FL produced by a genetically modified strain of *E. coli* K-12 DH1, have also been assessed by EFSA with positive outcomes (EFSA NDA Panel, 2022c,d, 2023a).

Since 2015, several scientific opinions with positive outcomes 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:

- Chemically synthetised 2′-FL (EFSA NDA Panel, 2015a) and 2′-FL produced by a genetically modified strain (APC199) of *C. glutamicum* ATCC 13032 (EFSA NDA Panel, 2022a);
- Chemically synthetised lacto-N-neotetraose (LNnT) (EFSA NDA Panel, 2015c) and LNnT produced by genetically modified strains of *E. coli* BL21 (DE3) (EFSA NDA Panel, 2020a);
- Extension of use in FS for children of chemically synthetised 2′-FL and LNnT (EFSA NDA Panel, 2015b) and extension of use in FS for infants of 2′-FL and LNnT produced by genetically modified strains of *E. coli* K-12 DH1 (EFSA NDA Panel, 2022c);
- Chemically synthetised N-acetyl-D-neuraminic acid (NANA) (EFSA NDA Panel, 2017);

[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, pp. 1–22.

[2] 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.

[3] Commission Implementing Decision (EU) 2016/376 of 11 March 2016 authorising the placing on the market of 2′-O-fucosyllactose as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council. OJ L 70, 16.3.2016, pp. 27–31.

[4] Commission Implementing regulation (EU) 2019/338 of 11 March 2019 authorising the change of the specifications of the novel food 2′-fucosyllactose produced with Escherichia coli K-12 under Regulation (EU) No 2015/2283 of the European Parliament and of the Council. OJ L 70, 12.3.2019, pp. 21–24.

[5] Commission Implementing regulation (EU) 2017/2201 of 27 November 2017 authorising the placing on the market of 2′-fucosyllactose produced with Escherichia coli strain BL21 as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council. OJ L 313, 29.11.2017, pp. 5–9.

[6] Commission Implementing Regulation (EU) 2023/859 of 25 April 2023 amending Implementing Regulation (EU) 2017/2470 as regards the specifications of the novel food 2′-Fucosyllactose (microbial source) to authorise its production by a derivative strain of *Corynebacterium glutamicum* ATCC 13032; OJ L 111, 25.4.2023, p. 17–22.

- 2′-FL/DFL mixture produced by a genetically modified strain of *E. coli* K-12 DH1 (EFSA NDA Panel, 2019a);
- Lacto-N-tetraose (LNT) produced by genetically modified strains of *E. coli* K-12 DH1 (EFSA NDA Panel, 2019b) or *E. coli* BL21 (DE3) (EFSA NDA Panel, 2022e);
- Extension of use in FS for infants of 2′-FL/DFL mixture and LNT produced by genetically modified strains of *E. coli* K-12 DH1 (EFSA NDA Panel, 2022d);
- 3-FL produced by genetically modified strains of *E. coli* K-12 MG1655 (EFSA NDA Panel, 2021), *E. coli* BL21 (DE3) (EFSA NDA Panel, 2022b) or *E. coli* K-12 DH1 (EFSA NDA Panel, 2023a);
- 6′-sialyllactose (6′-SL) sodium salts produced by genetically modified strains of *E. coli* K-12 DH1 (EFSA NDA Panel, 2020b) or *E. coli* BL21 (DE3) (EFSA NDA Panel, 2022f), or by *E. coli* NEO6, a genetically modified strain of the same parental strain *E. coli* W (ATCC 9637) (EFSA NDA Panel, 2023b);
- 3′-sialyllactose (3′-SL) sodium salts produced by genetically modified strains of *E. coli* K-12 DH1 (EFSA NDA Panel, 2020c) or *E. coli* BL21 (DE3) (EFSA NDA Panel, 2022g), or by *E. coli* NEO3, a genetically modified strain of the same parental strain *E. coli* W (ATCC 9637) (EFSA NDA Panel, 2023c).

2. Data and methodologies

2.1. Data

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

Administrative and scientific requirements for NF applications referred to in Article 10 of Regulation (EU) 2015/2283 are listed in Commission Implementing Regulation (EU) 2017/2469[7].

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 (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: (i) identity of the NF; (ii) production process; (iii) information on the genetically modified production strain; (iv) composition and stability of the NF; (v) toxicological and allergenicity studies.

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 Commission Implementing Regulation (EU) 2017/2469. The legal provisions for the assessment of food intended for infants and young children, food for special medical purposes (FSMP) and total diet replacement for weight control are laid down in Regulation (EU) No 609/2013[8] and, respectively, in Commission Delegated Regulation 2017/1798[9] (total diet replacement for weight control), in Commission Delegated Regulation (EU) 2016/128[10] (FSMP) and in Commission

[7] 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.

[8] 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 Directive92/52/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.

[9] Commission Delegated Regulation (EU) 2017/1798 of 2 June 2017 supplementing Regulation (EU) No 609/2013 of the European Parliament and of the Council as regards the specific compositional and information requirements for total diet replacement for weight control. OJ L 259, 7.10.2017, pp. 2–10.

[10] 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.

Delegated Regulation (EU) 2016/127[11] (as regards the specific compositional and information requirements for infant formula (IF) and follow-on formula (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. 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 2′-FL as primary constituent (≥ 82.0% w/w dry matter (DM)), a fucosylated neutral trisaccharide consisting of L-fucose linked via an α-(1-2′) bond to the D-galactose moiety of D-lactose. 2′-FL has been reported in most of the studies, as the most abundant component within the complex fraction of oligosaccharides naturally occurring in human milk (HMO), in the general population of breastfeeding women (Erney et al., 2000, 2001). The concentration of 2′-FL in human milk depends on the lactation period, with higher levels reported in the colostrum (Thurl et al., 2017). The Panel notes that although 2′-FL is the major component of the NF, related substances, namely D-lactose, L-fucose, fucosylgalactose, DFL, D-glucose and D-galactose, and a small fraction of other related saccharides, are also present. The NF is produced by fermentation by *E. coli* SGR5, a genetically modified strain of *E. coli* W (ATCC 9637).

The applicant applies for the same use and use levels as those already authorised for 2′-FL produced by chemical synthesis or fermentation by genetically modified strains of *E. coli* K-12 DH1, *E. coli* BL21 (DE3) or *Corynebacterium glutamicum* ATCC 13032 and included in the Union list of NFs (Commission Implementing Regulation 2017/2470[2]). The target population is the general population. The applicant also proposes to extend the use of 2′-FL in FS for infants at the use level of 1.2 g/day. In young children, the use level of 1.2 g/day is already authorised.

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

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 2′-FL (≥ 82.0% w/w DM), but it also contains D-lactose (≤ 5.0% w/w DM), L-fucose (≤ 1.0% w/w DM), fucosylgalactose (≤ 3.0% w/w DM), DFL (≤ 3.0% w/w DM), D-glucose and D-galactose (≤ 1.0% w/w DM, sum of both) and a small fraction of other related saccharides (sum of other carbohydrates ≤ 8.0% w/w DM). It is produced by fermentation by a genetically modified strain (*E. coli* SGR5) of *E. coli* W (ATCC 9637). 2′-FL is a trisaccharide consisting of L-fucose linked via an α-(1–2′) bond to the D-galactose moiety of D-lactose (Table 1 and Figure 1). 2′-FL is a constitutional isomer of 3-FL, which contains the same monosaccharide moieties as those present in 2′-FL but with an α-(1–3) bond between L-fucose and the D-glucose moiety of D-lactose.

[11] 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.

Table 1: Chemical identity of 2′-FL

Chemical substance	
Chemical (IUPAC) name	(2R,3R,4R,5R)-4-[(2S,3R,4S,5R,6R)-4,5-dihydroxy-6-(hydroxymethyl)-3-[(2S,3S,4R,5S,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxyoxan-2-yl]oxy-2,3,5,6-tetrahydroxyhexanal
Common name	2′-Fucosyllactose
Abbreviations	2′-FL
Alternative chemical names	• α-L-Fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→4)-D-glucopyranose • 2′-Fucosyl-D-lactose • 2′-O-fucosyllactose
CAS Number	41263–94–9
Molecular formula	$C_{18}H_{32}O_{15}$
Molecular mass	488.44 Da

CAS: Chemical Abstracts Service; IUPAC: International Union of Pure and Applied Chemistry.

Several analyses were performed on the NF in order to confirm the structure of 2′-FL, the major constituent of the NF.

The structure of 2′-FL[12] was determined by mono-dimensional (1D) nuclear magnetic resonance (NMR) spectroscopy, including ^{1}H and ^{13}C spectra, and two-dimensional (2D) NMR spectroscopy, including COSY (correlation spectroscopy), TOCSY (total correlation spectroscopy), HETCOR (heteronuclear correlation) and HMBC (heteronuclear multiple bond correlation) spectra, by comparison to a commercially available authentic specimen[13]. The relevant coupling constants measured by ^{1}H NMR together with the correlations evidenced on the 2D NMR spectra confirmed: (i) the α-(1″-2′) bond between L-fucose and the D-galactose moiety of D-lactose; (ii) the β-(1′-4) link between the D-galactose (Gal-C-1′) and D-glucose (Glc-C-4) moieties of D-lactose; and (iii) the β configuration of the Gal unit.

The molecular structure of 2′-FL[12] was corroborated by liquid chromatography-tandem mass spectrometry (LC-MS/MS) based on its retention factor (R_f) and fragmentation pattern, by comparison to a commercially available high-purity analytical standard, which allowed to differentiate between 2′-FL α-(1″-2′) and 3-FL α-(1″-3).

The identity of 2′-FL[12] was also corroborated by high-performance liquid chromatography-pulsed amperometric detection (HPLC-PAD) by comparison to a commercially available high-purity analytical standard.

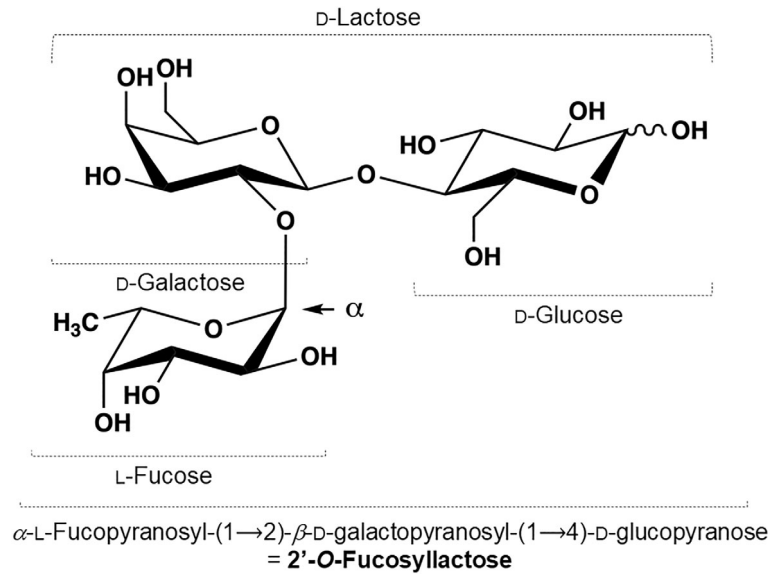

Figure 1: Chemical structure of 2′-FL (EFSA NDA Panel, 2022a)

[12] Batches of the NF produced with and without soy peptone in the fermentation media.
[13] 2′-FL isolated from human milk.

On the basis of the spectroscopic and chromatographic evidence, the Panel considers that the 2′-FL present in the NF produced by *E. coli* SGR5 is identical to the 2′-FL in human milk, and therefore, it is regarded as being a HiMO.

3.3. Production process

According to the information provided, the NF is produced in line with good manufacturing practice (GMP) and Hazard Analysis Critical Control Points (HACCP) principles, in a facility that is FSSC (Food Safety System Certification) 22000 certified.

The NF is produced by fermentation by a genetically modified strain (*E. coli* SGR5) of *E. coli* W (ATCC 9637) using food-grade raw materials and processing aids. The production microorganism is cultured under sterile conditions in a chemically defined nutrient medium and uses glucose and lactose to synthesise 2′-FL, which is excreted into the medium. Although certain batches of the NF used for safety assessment were produced with soy peptone in the fermentation medium, soy peptone is not present in the fermentation medium for commercial production purposes. The production microorganism is removed from the culture medium by microfiltration at the end of the fermentation process. 2′-FL is isolated and purified from the fermentation medium using a series of filtration and cationic and anionic exchange chromatography steps, followed by concentration and spray-drying to obtain the final 2′-FL product in powder form.

The production strain *E. coli* SGR5 is a genetically modified derivative of the parental strain *E. coli* W (Waksman's strain), which is deposited at the American Type Culture Collection (ATCC) (commercially available under ATCC 9637). The strain *E. coli* W is well characterised and its genome has been sequenced, annotated and compared to other safe *E. coli* strains and phylogroup B1 commensal/pathogenic *E. coli* strains (Archer et al., 2011). Although *E. coli* W harbours genes that encode pathogenicity determinants, these have been mutationally inactivated or are missing key components required for pathogenicity, similar to other safe strains (Archer et al., 2011). Genomic analyses also confirmed the lack of genes encoding toxins that can be secreted (Archer et al., 2011). Although the species *E. coli* is considered not suitable for qualified presumption of safety (QPS) status (EFSA BIOHAZ Panel, 2023), the strain *E. coli* W does not cause disease, does not colonise the human gut (Archer et al., 2011; Bauer et al., 2008; NIH, 2019), and is considered as a safe, non-pathogenic and non-toxigenic microorganism widely used for biotechnological applications.

The production strain has been deposited at the Japanese National Biological Resource Center (NBRC) culture collection. A detailed description of the genetic modification steps applied to the parental strain *E. coli* W to obtain the production strain *E. coli* SGR5 has been provided by the applicant. No residual DNA from the production strain was detected in the NF by a quantitative polymerase chain reaction (qPCR) assay using primers specific to the production strain. The absence of both DNA and viable cells from the production strain in the NF 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

In order to confirm that the manufacturing process is reproducible and adequate to produce on a commercial scale a product with certain characteristics, the applicant provided analytical information for nine batches of the NF produced with (six batches) or without (three batches) soy peptone in the fermentation media, the latter representing the conditions for commercial production of the NF (Table 2). Information was provided on the accreditation of the laboratories that conducted the analyses presented in the application.

Batch-to-batch analyses showed that the NF consists of 2′-FL as main component (92.8% w/w DM[14]/92.7% w/w DM[15] in batches produced with/without soy peptone in the fermentation media, respectively). The remaining constituents[16] include D-lactose (2.5% w/w DM[14]/1.1% w/w DM[15]), L-fucose

[14] Average content in six batches of the NF produced with soy peptone in the fermentation media.
[15] Average content in three batches of the NF produced without soy peptone in the fermentation media.
[16] 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.

(0.1% w/w DM[14]/≤ 0.05% w/w DM[15]), fucosylgalactose (0.6% w/w DM[14]/1.0% w/w DM[15]), DFL (1.0% w/w DM[14]/0.4% w/w DM[15]), D-glucose and D-galactose (0.1% w/w DM[14] / 0.3% w/w DM[15] – sum of both carbohydrates) and a small fraction of other related saccharides (sum of other carbohydrates,[17] 2.8% w/w DM[14]/4.4% w/w DM[15]).

With regard to physico-chemical properties, the NF can be described as a white to off-white powder. The solubility in water was measured in two batches of the NF produced with soy peptone and three batches without soy peptone in the fermentation media, according to the EFSA Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles (EFSA Scientific Committee, 2021), resulting in an average value of 446 g/L and 445 g/L, respectively.

[17] Sum of other carbohydrates = 100% w/w DM – 2′-FL (% w/w DM) – quantified carbohydrates (i.e. D-lactose, L-fucose, D-glucose and D-galactose, fucosylgalactose and DFL; % w/w DM) – ash (% w/w DM).

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

Parameters	NF (produced with soy peptone in the fermentation media)						NF (produced without soy peptone in the fermentation media)			Method of analysis
	#1	#2	#3	#4	#5	#6	#7	#8	#9	
Composition										
2′-FL (% w/w DM)	92.0	92.0	92.0	91.0	96.0	94.0	94.0	93.0	91.0	HPLC-PAD (validated internal method)
D-Lactose (% w/w DM)	3.1	2.7	2.3	2.4	2.1	2.3	1.1	1.1	1.1	HPLC-PAD[(a)] (validated internal method)
L-Fucose (% w/w DM)	≤ 0.05	0.1	0.1	0.1	0.1	0.1	≤ 0.05	≤ 0.05	≤ 0.05	HPLC-PAD[(a)] (validated internal method)
Fucosylgalactose (% w/w DM)	0.8	0.5	0.4	0.9	0.1	0.8	1.0	1.0	1.0	HPLC-PAD[(a)] (validated internal method)
DFL (% w/w DM)	0.5	1.4	0.9	1.0	1.1	1.0	0.4	0.4	0.4	HPLC-PAD[(a)] (validated internal method)
Sum of D-glucose and D-galactose (% w/w DM)	0.2	0.1	0.1	0.2	≤ 0.05	0.1	0.3	0.3	0.3	HPLC-PAD[(a),(b)] (validated internal method)
Sum of other carbohydrates (% w/w DM)	3.2	3.1	4.1	4.4	0.5	1.6	3.1	4.0	6.1	Calculation[(c)]
Water (% w/w)	5.0	3.9	3.9	2.7	2.8	2.3	4.7	5.3	4.5	JP 2.48[(d)] – Karl Fischer titration (volumetric/coulometric titration)
Ash (% w/w DM)	0.2	0.1	0.1	≤ 0.03	0.1	0.1	0.1	0.2	0.1	JP 2.44[(d),(e)] (residue on ignition, gravimetry)
Protein (% w/w)	≤ 0.01	–	–	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01	Bradford assay[(f)] (spectrophotometry)
pH (5% solution, 25°C)	6.3	6.4	6.2	5.7	6.1	6.2	6.1	6.0	6.2	JP 2.54[(d)] (potentiometry)
Contaminants										
Arsenic (mg/kg)	≤ 0.05	≤ 0.05	≤ 0.05	≤ 0.05	≤ 0.05	≤ 0.05	< 0.01[(i)]	< 0.01[(i)]	< 0.01[(i)]	USP 233[(g),(h)] (ICP-MS) AOAC (2019) 999.10 and 2011.14[(i)] (AAS and ICP-OES)
Cadmium (mg/kg)	≤ 0.05	≤ 0.05	≤ 0.05	≤ 0.05	≤ 0.05	≤ 0.05	< 0.01[(j)]	< 0.01[(j)]	< 0.01[(j)]	USP 233[(g),(h)] (ICP-MS) AOAC (2019) 999.10 and 2011.14[(j)] (AAS and ICP-OES)

Parameters	NF (produced with soy peptone in the fermentation media)						NF (produced without soy peptone in the fermentation media)			Method of analysis
	#1	#2	#3	#4	#5	#6	#7	#8	#9	
Lead (mg/kg)	≤ 0.05	≤ 0.05	≤ 0.05	≤ 0.05	≤ 0.05	≤ 0.05	< 0.02[k]	< 0.02[k]	< 0.02[k]	USP 233[g],[h] (ICP-MS) AOAC (2019) 999.10 and 2011.14[k] (AAS and ICP-OES)
Mercury (mg/kg)	≤ 0.05	≤ 0.05	≤ 0.05	≤ 0.05	≤ 0.05	≤ 0.05	< 0.004[l]	< 0.004[l]	< 0.004[l]	USP 233[g],[h] (ICP-MS) US EPA, February 2007, Method 7473[l] (AAS)
Aflatoxin M1 (μg/kg)	≤ 0.02	≤ 0.02	–	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	AOAC 2000.08[m] (HPLC)
Microbial parameters										
Total plate count (CFU/g)	< 40	< 40	< 10	110	< 10	< 10	< 10	< 10	< 10	ISO 4833-1:2013[n] (colony count)
Yeasts and moulds (CFU/g)	< 100	< 100	< 100	< 100	< 100	< 100	< 10[o]	< 10[o]	< 10[o]	ISO 21527-2:2008[p] (colony count)
Enterobacteriaceae (in 10 g)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ISO 21528-1:2017 (detection or qualitative method)
Salmonella spp. (in 25 g)	ND	–	–	ND	ND	ND	ND	ND	ND	ISO 6579-1:2017 (detection or qualitative method)
Cronobacter spp. (in 10 g)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ISO 22964:2017 (detection or qualitative method)
Listeria monocytogenes (in 25 g)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ISO 11290-1:2017 (detection or qualitative method)
Presumptive *Bacillus cereus* (CFU/g)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	ISO 7932:2004[q] (colony count)
Endotoxins (EU/mg)	0.0092	0.0005	0.0080	0.0028	0.0005	0.0003	< 0.0002[r]	< 0.0002[r]	< 0.0002[r]	JP17 4.01[d] (kinetic-turbidimetric method)

'–': Not reported; 2′-FL: 2′-Fucosyllactose; AAS: Atomic absorption spectroscopy; AOAC: Association of Official Analytical Collaboration; CFU: Colony forming units; DFL: Difucosyllactose; DM: Dry matter; EU: Endotoxin units; HPLC-PAD: High-performance liquid chromatography – pulsed amperometric detection; ICP-MS: Inductively coupled plasma – mass spectrometry; ICP-OES: Inductively coupled plasma – optical emission spectroscopy; ISO: International Organisation for Standardisation; JP: Japanese Pharmacopeia; LOD: Limit of detection; LOQ: Limit of quantification; ND: Not detected; US EPA: United States Environmental Protection Agency; USP: United States Pharmacopeia; w/w: Weight per weight.

(a): LOQ = 0.05% w/w DM.

(b): D-Glucose and D-galactose peaks on the HPLC-PAD chromatograms overlap.

(c): Sum of other carbohydrates (% w/w DM) = 100% w/w DM – 2′-FL (% w/w DM) – quantified carbohydrates (i.e. D-lactose, L-fucose, D-glucose and D-galactose, fucosylgalactose and DFL; % w/w DM) – ash (% w/w DM).

(d): Consistent with the compendial method specified in the 17th edition of the Japanese Pharmacopeia (2016).

(e): LOD ash = 0.03% w/w; Ash (% w/w DM) = ash (% w/w, wet)/(100 – water) × 100%.

(f): Evaluated using a limit test at 0.01% w/w.
(g): Consistent with the compendial method specified in the United States Pharmacopeia 35th revision (2011).
(h): LOQ for arsenic, cadmium, lead and mercury = 0.05 mg/kg.
(i): Method based on AOAC (2019) 999.10 and 2011.14. LOD = 0.01 mg/kg. LOQ = 0.03 mg/kg.
(j): Method based on AOAC (2019) 999.10 and 2011.14. LOD = 0.01 mg/kg. LOQ = 0.03 mg/kg.
(k): Method based on AOAC (2019) 999.10 and 2011.14. LOD = 0.02 mg/kg. LOQ = 0.03 mg/kg.
(l): Method based on U.S. EPA, February 2007, Method 7473, Mercury Analyser. LOD = 0.004 mg/kg. LOQ = 0.01 mg/kg.
(m): LOQ = 0.02 μg/kg.
(n): LOD = 10 CFU/g. In accordance with ISO 4833-1:2013, the presence of 1–3 colonies should be reported as < 40 CFU/g.
(o): LOD = 10 CFU/g (in-depth plating).
(p): LOD = 100 CFU/g (surface plating).
(q): LOD = 10 CFU/g.
(r): LOQ = 0.0002 EU/mg.

The Panel considers that the presence of soy peptone in the fermentation medium does not change the composition of the NF substantially. The Panel considers that the information provided on the composition is sufficient for characterising the NF.

3.4.1. Stability

Stability of the NF

The applicant provided interim results for a 3-year (real-time) stability study at 25°C and 60% relative humidity (RH) on one batch of the NF produced with soy peptone in the fermentation media, including measurements of the 2′-FL, carbohydrate and water content, and physico-chemical parameters (pH, appearance, colour) up to 30 months, and water activity measurements after 6, 12 and 24 months. In addition, a 6-month accelerated stability study at 40°C and 75% RH was conducted on five batches of the NF produced with soy peptone in the fermentation media, including the above-mentioned parameters (water activity measured after 6 months of storage). Microbial parameters were monitored on one batch of the NF (produced with soy peptone in the fermentation media) after 30 months under normal storage conditions and on two batches of the NF (produced with soy peptone in the fermentation media) after 6 months under accelerated storage conditions.

No significant changes in 2′-FL, carbohydrate and water content, physico-chemical parameters and water activity were observed over the storage period under normal and accelerated conditions. Microbial parameters were also below the respective limits of detection after 30 and 6 months of storage under normal and accelerated conditions, respectively. The applicant proposed a 30-month shelf-life under ambient conditions for the NF.

All stability studies were performed with batches of the NF produced with soy peptone in the fermentation media. However, since the presence of soy peptone in the fermentation media did not affect the composition of the NF substantially and is not expected by the Panel to affect the stability, the Panel considers that the available data provided sufficient information with respect to the stability of the NF for 30 months.

Stability of the NF under the intended conditions of use

No stability data for 2′-FL in food matrices were provided.

The applicant referred to the stability of 2′-FL in food matrices representative of the authorised food uses (EFSA NDA Panel, 2015a), including IF (for 3 years at 4, 20, 30 and 37°C), yoghurt (for 21 days at 4°C), citrus juice (for 28 days at 4°C) and ready-to-drink flavoured milk (for 14 days (pasteurised) or 28 days (ultra-high temperature (UHT) treated) at 4°C).

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

3.5. Specifications

The specifications of the NF are indicated in Table 3.

Table 3: Specifications of the NF

Description: 2′-fucosyllactose (2′-FL) is a white to off-white powder produced by microbial fermentation and further isolated, purified and concentrated.	
Source: A genetically modified strain (*Escherichia coli* SGR5) of *E. coli* W (ATCC 9637).	
Parameter	**Specification**
Composition	
2′-FL (% w/w DM)	≥ 82.0
D-Lactose (% w/w DM)	≤ 5.0
L-Fucose (% w/w DM)	≤ 1.0
Fucosylgalactose (% w/w DM)	≤ 3.0
DFL (% w/w DM)	≤ 3.0
Sum of D-glucose and D-galactose[(1)] (% w/w DM)	≤ 1.0
Sum of other carbohydrates[(2)] (% w/w DM)	≤ 8.0
Water (% w/w)	≤ 9.0

Parameter	Specification
Protein (% w/w)	≤ 0.01
Ash (% w/w)	≤ 0.5
pH (5% solution, 25°C)	4.5–8.5
Contaminants	
Arsenic (mg/kg)	≤ 0.2
Lead (mg/kg)	≤ 0.02
Cadmium (mg/kg)	≤ 0.1
Mercury (mg/kg)	≤ 0.1
Aflatoxin M1 (μg/kg)	≤ 0.025
Microbiological parameters	
Total plate count (CFU/g)	≤ 1,000
Yeasts and moulds (CFU/g)	≤ 100
Enterobacteriaceae (in 10 g)	ND
Salmonella (in 25 g)	ND
Cronobacter spp. (in 10 g)	ND
Listeria monocytogenes (in 25 g)	ND
Presumptive *Bacillus cereus* (CFU/g)	≤ 50
Endotoxins (EU/mg)	≤ 10

2′-FL: 2′-Fucosyllactose; CFU: Colony forming units; DFL: Difucosyllactose; DM: Dry matter; EU: Endotoxin units; ND: Not detected; w/w: Weight per weight.
(1): D-Glucose and D-galactose peaks on the HPLC-PAD chromatograms overlap.
(2): Sum of other carbohydrates = 100% w/w DM – 2′-FL (% w/w DM) – quantified carbohydrates (i.e. D-lactose, L-fucose, D-glucose and D-galactose, fucosylgalactose and DFL; % w/w DM) – ash (% w/w DM).

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, 2′-FL, which is the major constituent of the NF, is already included in the Union list of NFs when manufactured by chemical synthesis or fermentation by genetically modified strains of *E. coli* K-12 DH1, *E. coli* BL21 (DE3) or *C. glutamicum* ATCC 13032. It is authorised to be added to a variety of food categories (e.g. dairy products, beverages), including foods for special groups (e.g. IF and FOF) and FS, excluding FS for infants (intended for individuals above 1 year of age).

2′-FL is the most represented oligosaccharide in human milk with mean of mean concentrations of 2.28 g/L and a maximum mean concentration of 4.28 g/L across studies according to the recent review of Soyyılmaz et al. (2021). The Panel also notes that due to the relatively wide concentration range of 2′-FL in human milk (up to 4.78 g/L – Thurl et al., 2017; 5.57 g/L – Austin et al., 2019 and 5.85 g/L Samuel et al., 2019), higher intakes may occur.

Considering these values as representative of the average concentration range found in mature human milk and considering the average and high daily intakes of human milk (800 and 1,200 mL, respectively) for infants from 0 to 6 months (EFSA NDA Panel, 2013) in a 6.7-kg body weight (bw) infant (EFSA Scientific Committee, 2012), the estimated natural intakes are reported in Table 4.

Table 4: Estimated daily intakes of 2′-FL from average (800 mL) and high (1,200 mL) daily intakes of human milk for infants of 6.7 kg bw, based on the mean of mean concentrations (2.28 g/L) and the maximum mean (4.28 g/L) concentration of 2′-FL in mature human milk (lactation days 15–90; Soyyılmaz et al., 2021)

	Daily intake of 2′-FL (mg/kg bw) from 800 mL/day of human milk		Daily intake of 2′-FL (mg/kg bw) from 1,200 mL/day of human milk	
	Mean of mean concentrations	Maximum mean concentration	Mean of mean concentrations	Maximum mean concentration
2′-FL	272	511	408	767

bw: body weight.

In bovine milk, oligosaccharides are 20 times less concentrated than in human milk and acidic oligosaccharides are the most abundant oligosaccharides (i.e. 6′-SL), while fucosylated ones (i.e. 2′-FL) are found at very small concentrations (Aldredge et al., 2013; Urashima et al., 2013).

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 intended uses and use levels proposed for the NF as a food ingredient are the same as those already assessed for 2′-FL manufactured by fermentation by genetically modified strains of *E. coli* K-12 DH1, *E. coli* BL21 (DE3) or *C. glutamicum* ATCC 13032.

However, the applicant proposes to extend the use of 2′-FL in FS for infants at the use level of 1.2 g/day. In young children, the use level of 1.2 g/day is already authorised, whose safety has positively been assessed by EFSA for 2′-FL produced by a genetically modified strain of *E. coli* K-12 DH1 (EFSA NDA Panel, 2019a). Currently, the use of 2′-FL in FS for infants is not authorised.

3.7.3. Anticipated intake of the NF

The conditions of use of the NF are the same as for the authorised 2′-FL, with the exception for the use in FS in infants.

Owing to the same uses and use levels as food ingredient as per the authorised 2′-FL are proposed, in principle a new assessment of the intake is not needed. However, the Panel noted that the available intake estimate is not recent (EFSA NDA Panel, 2015a) and based on a different database (UK National Diet and Nutrition Survey (NDNS) for years 2008–2010) than that used by EFSA. For this reason, the Panel decided to perform a new intake estimate according to the current standard EFSA approach by using the EFSA Dietary Exposure (DietEx) tool,[18] which is based on 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 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.

Table 5: Intake estimate of the NF from the already authorised conditions of use

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	51	438	132	1,377
Young children[(c)]	1–< 3	116	398	292	869
Other children	3–< 10	45	198	140	605

[18] https://www.efsa.europa.eu/it/science/tools-and-resources/dietex

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)]
Adolescents	10–< 18	14	81	52	233
Adults[(d)]	≥ 18	36	153	95	337

(a): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 05 July 2023. 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 05 July 2023. The lowest and the highest P95 observed among all EU surveys are reported in these columns (P95 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.

According to EFSA's current intake assessment approach using the DietEx tool, the highest P95 daily intake of the NF from the already authorised conditions of use as an ingredient (Table 5) is higher than the estimated natural highest mean daily intake (Table 4) in infants and marginally higher in the young children.

Anticipated intake of the NF from the use in food supplements

The applicant has proposed a maximum daily intake of 1.2 g of the NF as FS for infants (up to 11 months). In young children (12–35 months), the use level of 1.2 g/day is already authorised.

In infants weighing 5 kg (EFSA Scientific Committee, 2012), this proposed maximum daily intake corresponds to 240 mg/kg bw. The Panel notes that the maximum daily intake of 2′-FL from the use of the NF in FS in infants is below the estimated daily intake of 2′-FL based on breast-fed infants from women with the mean of mean concentrations of 2′-FL (Table 4).

According to the applicant, FS containing the NF are not intended to be used if other sources of 2′-FL, human milk included, are consumed on the same day.

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

No absorption, distribution, metabolism and excretion (ADME) data were provided for the NF.

As mentioned by the applicant and reported in previous EFSA opinions (e.g. EFSA NDA Panel, 2015a, 2022c), HMOs, including fucosyllactoses, are considered 'non-digestible oligosaccharides' (EFSA NDA Panel, 2014) since they do not undergo any significant digestion by human enzymes in the upper gastrointestinal tract and that only small amounts are expected to be absorbed. Milk oligosaccharides are fermented in the colon by intestinal microbiota with a fraction excreted unchanged in the faeces and a small fraction found in the urine (EFSA NDA Panel, 2022a).

Finally, there are no indications that the absorption of 2′-FL, or other structurally related mono- and oligosaccharides (e.g. difucosyllactose) from the NF, differs from that of similar components in human milk.

3.9. Nutritional information

The NF is mainly composed of the non-digestible oligosaccharide 2′-FL.

The NF contains other carbohydrates individually present at low concentrations (slightly above or < 1%, see Table 2). D-Lactose is the most abundant molecule in human milk (~ 7%) and its monomers, D-glucose and D-galactose, are normal constituents of human milk. L-Fucose, which is present in traces, is a building block of the HMO. DFL also belongs to the group of fucosylated HMOs, which constitute on average about 70% of the total HMO fraction in human milk (Bode, 2012). Fucosylgalactose is a natural fucosylated component produced by fucosyltransferase activity (Bosmann et al., 1968).

The Panel considers that taking into account the composition of the NF and the proposed conditions of use, consumption of the NF is not nutritionally disadvantageous.

3.10. Toxicological information

The applicant provided four toxicological studies on the NF, which were conducted in compliance with OECD principles of good laboratory practices (GLP) (Organisation for Economic Co-operation and

Development principles of Good Laboratory Practices [OECD, 1998a,b]) and in accordance with the OECD test guidelines TG No 471, 474, 487 and 408. These studies which were claimed proprietary by the applicant are listed in Table 6.

Table 6: List of toxicological studies with the NF

Reference	Type of study	Test system	Dose
Study No. AG190035 (Unpublished, 2019a)	Bacterial reverse mutation test (GLP, OECD TG 471 (1997))	*Salmonella* Typhimurium TA98, TA100, TA1535 and TA1537 and *E. coli* WP2 *uvrA*	Up to 5,000 μg/plate (absence or presence of metabolic activation)
Study No. 190073 (Unpublished, 2019b)	Micronucleus study in bone marrow cells of mice (GLP, OECD TG 474 (2014a))	Male mouse ICR[S1c:ICR], SPF	500, 1,000 and 2,000 mg/kg
Study No. CG220002 (Unpublished, 2022)	*In vitro* micronucleus test (GLP, OECD TG 487 (2014b))	Mammalian cells (CHL/IU)	Up to 2,000 μg/mL (absence or presence of metabolic activation)
Study No. 109521RG (Unpublished, 2020)	90-day repeated oral toxicity study in rats (GLP, OECD TG 408 (1998b))	Rat, Crl:CD(SD)	500, 1,000 and 2,000 mg/kg

CHL/IU: Chinese hamster lung cell line; GLP: good laboratory practice; ICR: Institute for Cancer Research; OECD: Organisation for Economic Co-operation and Development; SD: Sprague Dawley; SPF: specific pathogen-free; TG: test guidelines; bw: body weight.

3.10.1. Genotoxicity

The potential genotoxicity of the NF was investigated in a bacterial reverse mutation test, in an *in vitro* mammalian cell micronucleus test and an *in vivo* micronucleus test in mice (Table 6).

The *in vitro* assessment of the mutagenic potential of the NF (Unpublished Study Report, 2019a) was performed with mutants of *S*. Typhimurium, strains TA98, TA100, TA1535 and TA1537, and a mutant of *E. coli* WP2 uvrA (pKM101). A mutagenicity test was conducted with the plate incorporation method at five different concentrations from 313 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 reproducible or dose-related increases in revertant colony numbers (less than twofold increase) over control counts were observed with any of the strains following exposure to 2′-FL at any concentration. No appreciable toxicity or precipitation was observed following exposure to any dose of the NF.

In the *in vitro* mammalian cell micronucleus test in Chinese hamster lung cell line (CHL/IU-Unpublished Study Report, 2022), concentrations of 2′-FL of 500, 1,000 and 2,000 μg/mL (main study) were tested after 6 or 27-h exposure in the presence (limited to 6 h) or absence of metabolic activation (S9 fraction). No cytotoxicity or precipitation has been observed and the percentage of micronuclei was not significantly increased in any of the test substance concentrations.

In addition, 2′-FL was also evaluated for its ability to induce micronuclei *in vivo* in the bone marrow of ICR mice (Unpublished Study Report, 2019b). Mice were treated orally with 2′-FL twice at a 24-h interval. Following a dose range finding study, doses of 500, 1,000 and 2,000 mg/kg body weight (bw) (main study) were tested in five male mice/dose. The incidence of micronucleated cells and the ratio of polychromatic erythrocytes to total erythrocytes in the NF-treated groups at about 24 h after the last administration was not statistically significantly different from the negative control group.

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. Subchronic toxicity

In the 90-day study, groups of 10 Crl:CD(SD) rats/sex were daily administered by gavage with a dose of 0, 500, 1,000 or 2,000 mg NF (96.6% purity)/kg bw (Unpublished Study Report, 2020).

There were no deaths in the course of the study and no treatment-related clinical signs were observed in any rats. Episodes of decreased food consumption were recorded in treated females (all dose levels) in comparison to the control group. Body weight and body weight gain were not affected by the treatment. No statistically significant changes in haematological and coagulation parameters were recorded. At clinical chemistry examination, statistically significant increase in glucose levels (males, low dose, about 11%) and in sodium levels (females, high dose, about 1%) was noted. A decrease in absolute weight of lungs (low dose, about 9%), pituitary (low and high dose, about 19%

and 13%, respectively) and kidneys (low dose, about 10%) in males and decrease in relative lungs weight (about 11%) in female rats (intermediate dose) was also noted (Appendix A). The Panel notes that the changes observed were of low magnitude and generally limited to only one sex or to low and intermediate doses and they are overall considered by the Panel as not biologically relevant.

At histological examination, no gross or histopathologic findings considered to be treatment related were noted. An increase in the incidence (unilateral or bilateral) of slight retinal dysplasia in both sexes and slight retinal atrophy in two female animals at high dose was recorded. Similar retinal findings have been recorded in concurrent control animals and in control animals in other subchronic studies from the same laboratory (EFSA NDA Panel, 2023b) and therefore considered by the Panel as not treatment related.

The Panel noted that the version of OECD TG 408 considered in the study (1998) is not the last version (2018) that was available at the start of the study. Therefore, data on thyroid hormone and some reproductive endpoints were not investigated.

The Panel considers that no adverse effects were observed in this study up to the highest tested dose of 2,000 mg NF/kg bw per day.

3.10.3. Human data

No human intervention studies with the NF were provided by the applicant.

In a recent publication (Schönknecht et al., 2023), a systematic review evaluated the health outcomes of 26 published clinical trials performed with HiMOs. A total of eight different HiMO alone or in mixtures at different dose levels in comparison with standard IF and/or breastfed infants were evaluated. 2′-FL appeared to be the most frequently used HiMO at concentrations ranging from 0.2 to 3 g/L in IF or up to 20 g/day as dietary supplement. The authors reported that overall in infants, HiMO supplementation 'was not associated with any serious adverse events'. Likewise, 'there were no safety or tolerance concerns in children and adults'.

Although 2′-FL of unknown production was used and sometimes administered together with other HiMOs or galacto- or fructo-oligosaccharides or probiotics, the Panel considers the information as supportive for the assessment of the NF.

3.11. Allergenicity

The applicant did not identify an allergenic potential of introduced proteins as a result of the genetic modification of the *E. coli* W (ATCC 9637) parental strain, assessed 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). The criterion used for identifying allergenic proteins was that of considering 'higher than 35% identity in a sliding window of 80 amino acids'.

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

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 2′-FL, but it also contains D-lactose, L-fucose, fucosylgalactose, DFL, D-glucose and D-galactose, and a small fraction of other related saccharides. The NF is produced by fermentation by a genetically modified strain (*E. coli* SGR5) of *E. coli* W (ATCC 9637).

The target population proposed by the applicant is the general population. The applicant intends to add the NF to a variety of foods, including IF and FOF, FSMP and FS. The applicant applies for the same uses and use levels as what is already authorised for 2′-FL, with the exception for the use as FS in infants for which the use of 2′-FL is not authorised. The applicant has proposed a maximum daily intake of 1.2 g of the NF as FS for infants (up to 11 months).

Since the available intake estimate for 2′-FL is not recent (EFSA NDA Panel, 2015a,b,c) and based on a different database (2008–2010 UK data), the Panel deemed it useful to conduct a new intake estimate according to the current EFSA approach and based on the relevant Comprehensive European Food Consumption Database (EFSA, 2011). The Panel notes that the highest P95 daily intake of the NF from the use as an ingredient is higher than the estimated natural highest mean daily intake in infants and marginally higher in the young children.

The newly proposed use as FS in infants of 1.2 g/day resulted in an estimated intake that is within the natural intake of 2-FL in breastfed infants (see Section 3.7.3). According to the applicant, FS containing the NF are not intended to be used if other sources of 2′-FL, including human milk for infants and young children, are consumed on the same day.

It is noted that additional sources for the oligosaccharides contained in the NF are cow milk and milk-derived products. However, the contribution from consumption of cow milk and milk-derived products is small (see Section 3.6.1).

The submitted toxicity studies did not raise safety concerns. No toxicologically relevant effects were observed in the subchronic toxicity study at up to the highest dose tested of 2,000 mg NF/kg bw per 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 breastfed 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.

5. Conclusions

The Panel concludes that the NF, which is composed of 2′-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 NMR spectroscopy, LC-MS/MS and HPLC-PAD; (ii) production process; (iii) information on the genetically modified production strain; (iv) composition and stability of the NF; (v) toxicological (Table 6) and allergenicity studies.

6. Steps taken by EFSA

1) On 07 December 2021 EFSA received a letter from the European Commission with the request for a scientific opinion on the safety of 2′-fucosyllactose (2′-FL) as a novel food. Ref. Ares(2021)7548426.
2) On 07 December 2021, a valid application on the safety of 2′-FL as a novel food, which was submitted by Kyowa Hakko Bio Co., Ltd, was made available to EFSA by the European Commission through the Commission e-submission portal (NF 2021/2394) and the scientific evaluation procedure was initiated.
3) On 10 June 2022, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4) On 30 June 2023, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
5) During its meeting on 26 September 2023, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of 2′-FL as a novel food pursuant to Regulation (EU) 2015/2283.

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Abbreviations

1D	Mono-dimensional
2D	Two-dimensional
2′-FL	2′-Fucosyllactose
3-FL	3-Fucosyllactose
3′-SL	3′-Sialyllactose
6′-SL	6′-Sialyllactose
AAS	Atomic absorption spectroscopy
ADME	Absorption, Distribution, Metabolism and Excretion
AOAC	Association of Official Analytical Collaboration
APC	Adenomatous polyposis coli
ATCC	American Type Culture Collection
BIOHAZ	EFSA Panel on Biological Hazards
bw	Body weight
CAS	Chemical Abstracts Service
CFU	Colony forming unit
CHL/IU	Chinese hamster lung cells line
COSY	Correlation spectroscopy
Crl:CD(SD) rats	Charles River Laboratories: Caesarean-derived (Sprague Dawley) rats
DFL	Difucosyllactose
DietEx	EFSA Dietary Exposure tool
DM	Dry matter
DNA	Deoxyribonucleic acid
EC	European Commission
EFSA	European Food Safety Authority
Escherichia coli W	Waksman's *E. coli* strain
EU	Endotoxin unit
EU	European Union
F	Females
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
FOF	Follow-on formula
FS	Food supplements
FSMP	Food for special medical purposes
FSSC 22000	Food Safety System Certification 22000
Gal	Galactose
Glc	Glucose
GLP	Good Laboratory Practices
GMO	EFSA Panel on Genetically Modified Organisms
GMP	Good Manufacturing Practices
HACCP	Hazard Analysis Critical Control Points
HETCOR	Heteronuclear correlation
HiMO	Human-identical milk oligosaccharide

HMBC	Heteronuclear multiple-bond correlation
HMO	Human milk oligosaccharide
HPLC-PAD	High performance liquid chromatography – pulsed amperometric detection
ICP-MS	Inductively coupled plasma – mass spectrometry
ICP-OES	Inductively coupled plasma – optical emission spectroscopy
ICR	Institute of Cancer Research
IF	Infant formula
ISO	International Organisation for Standardisation
IUPAC	International Union of Pure and Applied Chemistry
JP	Japanese Pharmacopoeia
LC	Liquid chromatography
LNnT	Lacto-N-neotetraose
LNT	Lacto-N-tetraose
LOD	Limit of detection
LOQ	Limit of quantification
M	Males
MS/MS	Tandem mass spectrometry
NANA, Neu5A	N-acetyl-D-neuraminic acid, sialic acid
NBRC	National Biological Resource Center
ND	Not detected
NDA	EFSA Panel on Nutrition, Novel Foods and Food Allergens
NDNS	UK National Diet and Nutrition Survey
NF	Novel food
NIH	National Institutes of Health
NMR	Nuclear magnetic resonance spectroscopy
OECD	Organisation for Economic Co-operation and Development
qPCR	Quantitative polymerase chain reaction
QPS	Qualified presumption of safety
R_f	Retention factor
RH	Relative humidity
SD	Standard deviation
SD rats	Sprague Dawley rats
SPF	Specific pathogen-free
TG	Test guidelines
TOCSY	Total correlation spectroscopy
UHT	Ultra-high temperature
US EPA	US Environmental Protection Agency
US FDA	US Food and Drug Administration
USP	US Pharmacopoeia
W	Wakman's strain
w/w	Weight per weight

Appendix A – Summary of the 90-day oral toxicity study in rats

Study title	**90-day oral toxicity study on 2′-FL in SD rats** (Unpublished study report, 2020)			
Key results				
Parameters	**Sex**	**Dose groups (expressed in mg 2′-FL/kg bw per day)**		
		0 (Control, G1); Mean ± SD	**500 (Low dose, G2); Mean ± SD**	**1,000 (Intermediate dose, G3); Mean ± SD**	**2,000 (High dose, G4); Mean ± SD**
Food consumption					
Study Day 14–15	M	27.1 ± 3.7	26.6 ± 2.7	25.6 ± 2.3	27.1 ± 1.7
	F	18.7 ± 1.5	17.4 ± 2.2	15.4 ± 1.6**	18.4 ± 2.5
Study Day 18–19	M	26.7 ± 2.0	25.6 ± 2.6	25.3 ± 3.1	24.8 ± 1.8
	F	16.9 ± 1.7	15.5 ± 1.9	14.4 ± 2.0*	16.2 ± 2.2
Study Day 35–36	M	25.7 ± 2.7	24.5 ± 4.5	25.3 ± 2.2	22.6 ± 2.5
	F	17.2 ± 2.5	15.2 ± 1.2	14.9 ± 2.0*	14.3 ± 1.9**
Study Day 63–64	M	25.2 ± 2.7	24.2 ± 3.1	25.4 ± 2.2	23.3 ± 1.5
	F	17.9 ± 4.1	14.8 ± 2.1*	14.5 ± 2.1*	15.8 ± 2.1
Clinical chemistry					
Glucose (mg/dL)	M	117 ± 5	130 ± 13*	122 ± 9	126 ± 11
	F	118 ± 20	117 ± 19	110 ± 16	107 ± 20
Sodium (mmol/L)	M	141 ± 1	141 ± 1	142 ± 1	141 ± 1
	F	140 ± 1	141 ± 2	141 ± 1	142 ± 1*
Organ weight values – absolute					
Lungs (g)	M	1.64 ± 0.13	1.50 ± 0.13*	1.63 ± 0.09	1.62 ± 0.10
	F	1.15 ± 0.09	1.20 ± 0.11	1.20 ± 0.07	1.14 ± 0.07
Pituitary (mg)	M	16.1 ± 2.3	13.0 ± 1.6**	15.2 ± 2.0	14.0 ± 0.9*
	F	20.2 ± 2.9	18.5 ± 2.3	20.0 ± 2.6	19.6 ± 3.5
Kidneys (g)	M	3.47 ± 0.29	3.13 ± 0.19*	3.43 ± 0.27	3.40 ± 0.29
	F	2.03 ± 0.18	2.09 ± 0.16	2.02 ± 0.14	2.13 ± 0.24
Organ weight values – relative to bw					
Lungs (g/g bw)	M	0.0029 ± 0.0002	0.0028 ± 0.0002	0.0029 ± 0.0002	0.0029 ± 0.0002
	F	0.0037 ± 0.0003	0.0040 ± 0.0002	0.0041 ± 0.0003*	0.0038 ± 0.0004

SD: standard deviation; M: males; F: females; bw: body weight.
*p < 0.05, **p < 0.01: significant difference in the parametric procedure.

Retinal findings in subchronic studies performed by Higashimatsuyama Laboratories (2020–2021)

90-day 2′-FL	**M**		**F**	
	Control	**High dose**	**Control**	**High dose**
Retinal dysplasia, unilateral	1++	2+	–	–
Retinal dysplasia, bilateral	1+	1+	–	2+*
Retinal atrophy, peripheral, unilateral	–	–	–	1+*
Retinal atrophy, peripheral, bilateral	–	–	–	1+

Unpublished study report (2020)
Higashimatsuyama Laboratories – Study No. 109521RG. 90-day repeated oral dose toxicity study of 2′-FL in rats

90-day 3′-SL	M		F	
	Control	High dose	Control	High dose
Retinal dysplasia, unilateral	1+	1+, 1++	2+, 1++	1+
Retinal dysplasia, bilateral	1+/++	–	–	–
Retinal atrophy, focal, unilateral	–	–	1+	1+
Retinal atrophy, peripheral, bilateral	–	–	–	–

Unpublished study report (2021)
Higashimatsuyama Laboratories – Study No. 100602RG. 90-day repeated oral dose toxicity study of 3′-SL sodium salt in rats (EFSA NDA Panel, 2023c)

90-day 6′-SL	M		F	
	Control	High dose	Control	High dose
Retinal dysplasia, unilateral	–	2+,1++	1++	–
Retinal dysplasia, bilateral	1+	–	–	–
Retinal atrophy, focal, unilateral	–	–	–	–
Retinal atrophy, peripheral, bilateral	–	–	–	–

Unpublished Study Report (2021)
Higashimatsuyama Laboratories – Study No. 100603RG. 90-day repeated oral dose toxicity study of 6′-SL sodium salt in rats (EFSA NDA Panel, 2023b)

Histology of the 'eye ball, including optic nerve'; + slight; ++ moderate; * the same rat.

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

Information provided in this Annex can be found in the online version of this output (in the 'Supporting information' section).