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SCIENTIFIC OPINION

# Safety of lacto-N-fucopentaose I/2′-fucosyllactose (LNFP-I/2′-FL) mixture 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 lacto-N-fucopentaose I (LNFP-I)/2′-fucosyllactose (2′-FL) mixture as a novel food (NF) pursuant to Regulation (EU) 2015/2283. The NF is mainly composed of the human-identical milk oligosaccharides (HiMO) LNFP-I and 2′-FL, but it also contains D-lactose, lacto-N-tetraose, difucosyllactose, 3-fucosyllactose, LNFP-I fructose isomer, 2′-fucosyl-D-lactulose, L-fucose and 2′-fucosyl-D-lactitol, and a small fraction of other related saccharides. The NF is produced by fermentation by a genetically modified strain (*Escherichia coli* K-12 DH1 MDO MP2173b) of *E. coli* K-12 DH1 (DSM 4235). The information provided on the identity, 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 formula (IF) and follow-on formula, foods for infants and toddlers, foods for special medical purposes and food supplements (FS). The target population is the general population. The anticipated daily intake of LNFP-I from use in IF is similar to the estimated natural mean highest daily intake in breastfed infants. Overall, the anticipated daily intake of LNFP-I from the NF as a food ingredient at the maximum proposed use levels is unlikely to exceed the intake level of breastfed infants on a body weight basis. The intake in breastfed infants on a body weight basis is expected to be safe also for other population groups. The anticipated 2′-FL intake is generally rather low. The use of the NF in FS is not intended if other foods with added NF components or human milk (for infants and young children) are consumed on the same day. The Panel concludes that the NF, a mixture of LNFP-I and 2′-FL, is safe under the proposed conditions of use.

**KEYWORDS**

2′-FL, 2′-fucosyllactose, HiMO, human milk oligosaccharide, lacto-N-fucopentaose I, LNFP-I, novel food

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# 1 | INTRODUCTION

## 1.1 | Background and Terms of Reference as provided by the requestor

On 1 March 2021, the company Glycom A/S submitted a request to the Commission in accordance with Article 10 of Regulation (EU) No 2015/2283[1] to place on the EU market, a lacto-N-fucopentaose I/2′-fucosyllactose (LNFP-I/2′-FL) mixture as a novel food (NF).

The NF is intended to be used in a number of foods, in foods for special medical purposes (FSMP) as defined by Regulation (EU) 609/2013[2] and in food supplements (FS) as defined in Directive 2002/46/EC[3].

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 the LNFP-I/2′-FL mixture as a NF.

In addition, EFSA is requested to include in its scientific opinion a statement as to if, and if so to what extent, the proprietary data for which the applicant is requesting data protection was used in elaborating the opinion in line with the requirements of Article 26(2)(c) of Regulation (EU) 2015/2283.

## 1.2 | Additional information

2′-FL is included in the Union list of authorised NFs (Commission Implementing Regulation (EU) 2017/2470[4]) when chemically synthesised (Commission Implementing Decision (EU) 2016/376[5]) (EFSA NDA Panel, 2015a, 2015b) or produced by fermentation by genetically modified strains of *Escherichia coli* K-12 DH1 (Commission Implementing Regulation (EU) 2019/338[6]), *E. coli* BL21 (DE3) (Commission Implementing Regulation (EU) 2017/2201[7]) or *Corynebacterium glutamicum* ATCC 13032 (Commission Implementing Regulation (EU) 2023/859[8]) (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 safety of 2′-FL produced by a genetically modified strain of *E. coli* W (ATCC 9637) (EFSA NDA Panel, 2023a), the extension of use in FS for infants of 2′-FL and a 2′-FL/DFL mixture, both produced by genetically modified strains of *E. coli* K-12 DH1 (EFSA NDA Panel, 2022c, 2022d), the extension of use of 2'-FL produced by a genetically modified strain of *E. coli* BL21 (DE3) (EFSA NDA Panel, 2023b) and the safety of 3-FL produced by a genetically modified strain of *E. coli* K-12 DH1 (EFSA NDA Panel, 2023c), have also been assessed by EFSA with positive outcomes.

LNFP-I is a fucosylated derivative of lacto-N-tetraose (LNT), which is authorised as a NF when produced by genetically modified strains of *E. coli* K-12 DH1 (EFSA NDA Panel, 2019b) or *E. coli* BL21 (DE3) (EFSA NDA Panel, 2022e). Moreover, the extension of use in FS for infants of LNT produced by a genetically modified strain of *E. coli* K-12 DH1 has been assessed by EFSA with a positive outcome (EFSA NDA Panel, 2022d).

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), 2′-FL produced by genetically modified strains (APC199) of *C. glutamicum* ATCC 13032 (EFSA NDA Panel, 2022a) or *E. coli* W (ATCC 9637) (EFSA NDA Panel, 2023a) and extension of use of 2'-FL produced by a genetically modified strain of *E. coli* BL21 (DE3) (EFSA NDA Panel, 2023b);
- 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);

[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]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.

[3]Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements. OJ L 183, 12.7.2002, p. 51–57.

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

[5]Commission Implementing 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.

[6]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.

[7]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.

[8]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.

- Chemically synthetised N-acetyl-D-neuraminic acid (NANA) (EFSA NDA Panel, 2017);
- 2′-FL/DFL mixture produced by a genetically modified strain of *E. coli* K-12 DH1 (EFSA NDA Panel, 2019a);
- 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, 2023c).
- 6′-sialyllactose (6′-SL) sodium salts produced by genetically modified strains of *E. coli* K-12 DH1 (EFSA NDA Panel, 2020b), *E. coli* BL21 (DE3) (EFSA NDA Panel, 2022f) or *E. coli* W (ATCC 9637) (EFSA NDA Panel, 2023d);
- 3′-sialyllactose (3′-SL) sodium salts produced by genetically modified strains of *E. coli* K-12 DH1 (EFSA NDA Panel, 2020c), *E. coli* BL21 (DE3) (EFSA NDA Panel, 2022g) or *E. coli* W (ATCC 9637) (EFSA NDA Panel, 2023e).

# 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[9].

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) intake assessment; and (vi) toxicological information.

## 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, FSMP and total diet replacement for weight control are laid down in Regulation (EU) No 609/2013[2] and, respectively, in Commission Delegated Regulation 2017/1798[10] (total diet replacement for weight control), in Commission Delegated Regulation (EU) 2016/128[11] (FSMP), and in Commission Delegated Regulation (EU) 2016/127[12] (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.

# 3 | ASSESSMENT

## 3.1 | Introduction

The NF, which is the subject of the application, is a mixture of LNFP-I and 2′-FL (75.0%–100.0% w/w dry matter (DM); 50.0%–75.0% LNFP-I and 15.0%–35.0% 2′-FL), both fucosylated neutral oligosaccharides. LNFP-I and 2′-FL are naturally occurring in mammalian milk, with the highest concentrations being found in human milk, thus being typically acknowledged as

[9]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.

[10]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.

[11]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.

[12]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.

human milk oligosaccharides (HMOs). With more than 200 different HMO structures (up to 15 core structures) being detected in human milk (Remoroza et al., 2020), LNFP-I and 2′-FL are among the five most abundant HMOs, which account, on average, for nearly half of the oligosaccharide mass fraction in human milk (Molnar-Gabor et al., 2019; Thurl et al., 2017).

The Panel notes that although LNFP-I and 2′-FL are the major components of the NF, it also contains D-lactose, LNT, DFL, LNFP-I fructose isomer, 3-FL, L-fucose and 2′-fucosyl-D-lactitol, 2′-fucosyl-D-lactulose and a small fraction of other related saccharides. The NF is produced by fermentation by *E. coli* K-12 DH1 MDO MP2173b, a genetically modified strain of *E. coli* K-12 DH1 (DSM 4235).

The NF is proposed to be used in IF, FOF, FSMP and total diet replacements for weight control, as defined in Regulation (EU) No 609/2013, FS as defined in Directive 2002/46/EC, beverages and in a variety of other foods (e.g. dairy products, cereals). The target population is the general population.

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 LNFP-I and 2′-FL (75.0%–100.0% w/w DM; 50.0%–75.0% LNFP-I and 15.0%–35.0% 2′-FL), but it also contains D-lactose (≤ 10.0% w/w), LNT (≤ 5.0% w/w), DFL (≤ 2.0% w/w), LNFP-I fructose isomer (≤ 1.5% w/w), 3-FL (≤ 1.0% w/w), 2′-fucosyl-D-lactulose (≤ 1.0% w/w) and L-fucose and 2′-fucosyl-D-lactitol (≤ 1.0% w/w, sum of both), and a small fraction of other related saccharides (sum of other carbohydrates ≤ 6.0% w/w). It is produced by fermentation by a genetically modified strain (*E. coli* K-12 DH1 MDO MP2173b) of *E. coli* K-12 DH1 (DSM 4235). LNFP-I is a fucosylated derivative of LNT, i.e. a pentasaccharide consisting of L-fucose linked to D-galactose via an α-(1–2) bond, which is linked through a β-(1–3) bond to N-acetyl-D-glucosamine (GlcNAc), linked through a β-(1–3) bond to D-galactose, linked through a β-(1–4) bond to the reducing end D-glucose (Table 1 and Figure 1). 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).

**TABLE 1** Chemical identity of LNFP-I and 2′-FL.

| **Chemical substance** | |
|---|---|
| Chemical (IUPAC) name | **LNFP-I:** N-[(2S,3R,4R,5S,6R)-2-[(2R,3S,4S,5R,6S)-3,5-dihydroxy-2-(hydroxymethyl)-6-[(2R,3S,4R,5R)-4,5,6-trihydroxy-2-(hydroxymethyl)oxan-3-yl]oxyoxan-4-yl]oxy-4-[(2R,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-5-hydroxy-6-(hydroxymethyl)oxan-3-yl]acetamide<br>**2′-FL:** (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 |
| IUPAC abbreviations | **Extended**<br>**LNFP-I:** α-L-Fuc*p*-(1–2)-β-D-Gal*p*-(1–3)-β-D-GlcNAc*p*-(1–3)-β-D-Gal*p*-(1–4)-Glc<br>**2′-FL:** α-L-Fuc*p*-(1–2)-β-D-Gal*p*-(1–4)-D-Glc<br>**Condensed**<br>**LNFP-I:** Fuc-(α1-2)-Gal-(β1-3)-GlcNAc-(β1-3)-Gal-(β1-4)-Glc<br>**2′-FL:** Fuc-(α1-2)-Gal-(β1-4)-Glc |
| Common name | Lacto-N-fucopentaose I/2′-fucosyllactose mixture |
| Synonyms | **LNFP-I:** LNF I<br>**2′-FL:** 2′-O-Fucosyllactose, 2′-Fucosidolactose, 2-FL |
| Abbreviations | LNFP-I/2′-FL mixture (LNFP-I/2′FL mixture; LNFPI/2FL mixture; LNF I/2′-FL mixture; LNF I/2′FL mixture; LNF I/2FL mixture) |
| CAS Number | **LNFP-I:** 7578-25-8<br>**2′-FL:** 41263-94-9 |
| Other IUPAC names | **LNFP-I:**<br>• α-L-Fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-D-glucopyranose<br>• O-6-Deoxy-α-L-galactopyranosyl-(1→2)-O-β-D-galactopyranosyl-(1→3)-O-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl-(1→)-O-β-D-galactopyranosyl-(1→4)-D-glucose<br>**2′-FL:**<br>• α-L-Fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→4)-D-glucopyranose<br>• O-6-Deoxy-α-L-galactopyranosyl-(1→2)-O-β-D-galactopyranosyl-(1→4)-D-glucose |
| Molecular formula | **LNFP-I:** $C_{32}H_{55}NO_{25}$<br>**2′-FL:** $C_{18}H_{32}O_{15}$ |
| Molecular weight | **LNFP-I:** 853.77 Da<br>**2′-FL:** 488.44 Da |

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

Several analyses were performed on the NF in order to confirm the structures of LNFP-I and 2′-FL, the major constituents of the NF.

The structure of LNFP-I was determined by mono-dimensional (1D) nuclear magnetic resonance (NMR) spectroscopy, including $^{1}H$, $^{13}C$ and $^{13}C$-DEPT-Q (distortionless enhancement by polarisation transfer with retention of quaternaries) spectra and two-dimensional (2D) NMR spectroscopy, including g-DQFCOSY (gradient double-quantum-filtered correlation spectroscopy), g-HSQC (gradient heteronuclear single quantum coherence), g-HMBC (gradient heteronuclear multiple bond coherence), TOCSY (total correlation spectroscopy) and NOESY (nuclear overhauser effect spectroscopy) spectra, by comparison to a commercially available authentic specimen[13]. The identity of the glycosidic bonds was verified by the 8.0 and 7.7 Hz values of the $J_{1,2}$ coupling constants in the D-galactose units indicating their β configurations; the 8.4 value Hz in the N-acetyl-D-glucosamine unit indicating its β configuration; and the 4.0 Hz value in the L-fucose unit indicating its α-configuration. The connections of the carbohydrate units are demonstrated by the long-range carbon-proton couplings and through space correlations (NOESY). The full assignment of the LNFP-I NMR spectra is consistent with relevant literature (Breg et al., 1988; Rao et al., 1985).

The molecular structure of 2′-FL was also demonstrated by mono-dimensional $^{1}H$ and $^{13}C$ NMR spectra, selective one-dimensional TOCSY spectra and two-dimensional HSQC and HMBC 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 structures of LNFP-I and 2′-FL were corroborated by liquid chromatography–tandem mass spectrometry (LC–MS/MS) based on its collision-induced dissociation (CID) fragmentation pattern by comparison to commercially available high-purity analytical standards. The mass fragmentation patterns are consistent with those reported in the literature (Chai et al., 2001, Pfenninger et al., 2002).

The identities of LNFP-I and 2′-FL were also corroborated by high-performance liquid chromatography–charged aerosol detection (HPLC-CAD) by comparison to commercially available high-purity analytical standards.

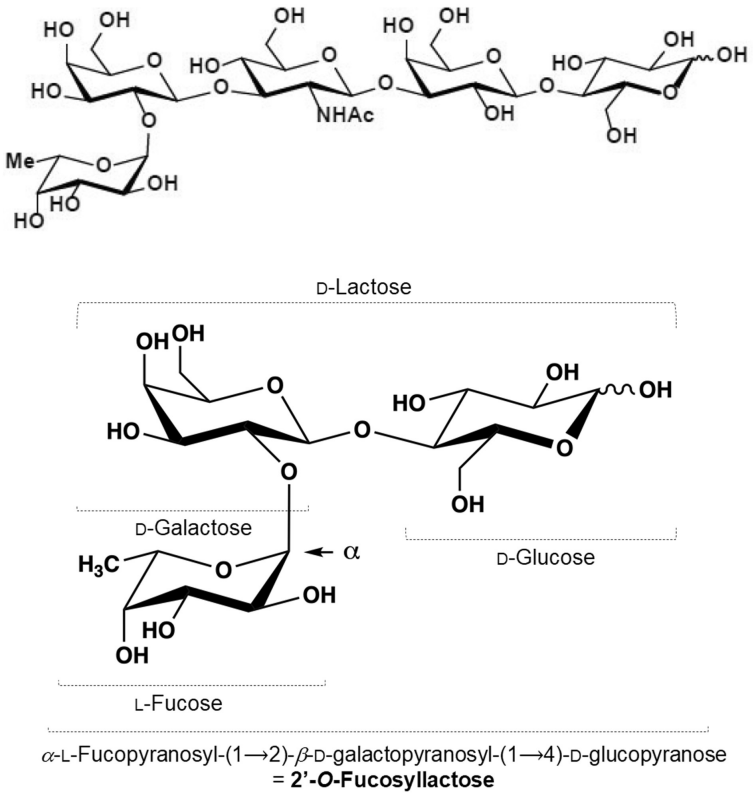


**FIGURE 1** Chemical structure of LNFP-I (top) and 2′-FL (bottom).

On the basis of the spectroscopic and chromatographic evidence, the Panel considers that the LNFP-I and 2′-FL present in the NF produced by *E. coli* K-12 DH1 MDO MP2173b are identical to the LNFP-I and 2′-FL in human milk and therefore, they are regarded as being HiMOs.

[13]LNFP-I and 2′-FL isolated from human milk.

## 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. The production process (including all used processing aids, raw materials, unit operations and filter aids), as well as food safety management system comply with the following standards and certifications: Food Safety Systems Certification (FSSC) 22000 and International Organisation for Standardisation (ISO) 9001.

The NF is produced by fermentation by a genetically modified strain (*E. coli* K-12 DH1 MDO MP2173b) of *E. coli* K-12 DH1. D-Lactose and D-glucose (alternatively, D-sucrose or glycerol) are converted to LNFP-I/2′-FL by the adapted cellular metabolism of the production strain, which uses D-glucose as an energy and carbon source and D-lactose as a substrate for the biosynthesis. The production microorganism is removed from the fermentation medium by ultrafiltration/diafiltration and microfiltration at the end of the fermentation process. A series of isolation, purification and concentration steps are then used to obtain high-purity LNFP-I/2′-FL in powder form.

The production strain *E. coli* K-12 DH1 MDO MP2173b is a genetically modified derivative of the parental strain *E. coli* K-12 DH1 (*F- ʎ- gyrA96 recA1 relA1 endA1 thi-1 hsdR17 supE44*), which was obtained by the applicant from the German Collection of Microorganisms and Cell Cultures (DSMZ) (commercially available under DSM 4235). The parental strain *E. coli* K-12 DH1 is derived from *E. coli* K-12 by forced random mutagenesis. The whole genomes of *E. coli* K-12 and other closely derivative strains, including *E. coli* K-12 DH1, were sequenced and compared to other *E. coli* strains including pathogenic strains, which evidenced genomic differences in *E. coli* K-12 and its derivatives as compared to the pathogenic strains (Blattner et al., 1997; Lukjancenko et al., 2010). Although the species *E. coli* is considered non suitable for qualified presumption of safety (QPS) status (EFSA BIOHAZ Panel, 2023), the strain *E. coli* K-12 is considered as a safe, non-pathogenic and non-toxigenic microorganism widely used for biotechnological applications (Gorbach, 1978; Muhldorfer & Hacker, 1994; OECD, 1986; US EPA, 1997; ZKBS, 2021).

The production strain has been deposited at the DSMZ culture collection. A detailed description of the genetic modification steps applied to the parental strain *E. coli* K-12 DH1 (DSM 4235) to obtain the platform strain *E. coli* K-12 DH1 MDO (membrane-derived oligosaccharides) and the production strain *E. coli* K-12 DH1 MDO MP2173b has been provided by the applicant. No residual DNA from the production strain was detected in the NF using three quantitative polymerase chain reaction (qPCR) assays targeting short sub-sequences of specific inserted genes, as well as a short sub-sequence of the 23S rRNA subunit of *E. coli*. 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 six batches 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 LNFP-I and 2′-FL as main components[14] (88.4% w/w DM, LNFP-I/2′-FL; 61.9% w/w DM LNFP-I; 26.5% w/w DM 2′-FL). The remaining constituents[14,15] include D-lactose (2.2% w/w), LNT (1.9% w/w), DFL (0.5% w/w), LNFP-I fructose isomer (0.3% w/w), 3-FL (0.04% w/w), L-fucose and 2′-fucosyl-D-lactitol (0.04% w/w, sum of both carbohydrates), 2′-fucosyl-D-lactulose (0.2% w/w) and a small fraction of other related saccharides (sum of other quantified carbohydrates, 1.9% w/w DM).

With regards to physico-chemical properties, the NF can be described as a white to off-white powder. The solubility in water of one batch of the NF was measured, 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 781 g/L. Therefore, the NF is considered as highly soluble.

[14]Average content in six batches of the NF.

[15]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.

**TABLE 2** Batch to batch analysis of the NF.

| Parameters | Batches of the NF | | | | | | Method of analysis |
|---|---|---|---|---|---|---|---|
| | #1 | #2 | #3 | #4 | #5 | #6 | |
| Composition | | | | | | | |
| Specified saccharides[a] (% w/w DM) | 92.65 | 94.04 | 93.28 | 93.82 | 92.39 | 96.14 | HPAEC-PAD, HPLC-CAD (validated internal methods) |
| LNFP-I and 2′-FL (% w/w DM) | 89.46 | 89.89 | 88.45 | 80.84 | 88.71 | 93.07 | |
| LNFP-I (% w/w DM) | 57.70 | 62.91 | 70.17 | 59.92 | 57.40 | 63.15 | |
| 2′-FL (% w/w DM) | 31.76 | 26.98 | 18.29 | 20.92 | 31.30 | 29.92 | |
| L-Fucose and 2′-fucosyl-D-lactitol[b] (% w/w) | < 0.03 | < 0.03 | < 0.03 | 0.11 | < 0.03 | < 0.03 | |
| D-Lactose (% w/w) | 0.44 | 1.42 | 0.72 | 8.56 | 0.89 | 0.91 | |
| 3-FL (% w/w) | 0.11 | < 0.03 | < 0.03 | 0.03 | < 0.03 | < 0.03 | |
| DFL (% w/w) | 0.70 | 0.48 | 0.28 | 0.19 | 0.66 | 0.68 | |
| LNT (% w/w) | 0.65 | 1.68 | 3.37 | 3.21 | 1.53 | 1.08 | |
| LNFP-I fructose isomer (% w/w) | 0.67 | 0.26 | 0.22 | 0.18 | 0.12 | 0.16 | |
| 2′-Fucosyl-D-lactulose (% w/w) | 0.60 | 0.18 | 0.11 | 0.18 | 0.24 | 0.15 | |
| Sum of other carbohydrates (% w/w) | 2.64 | 1.43 | 1.51 | 1.53 | 2.72 | 1.51 | |
| pH (5% solution, 20°C) | 4.6 | 5.9 | 5.7 | 5.4 | 4.4 | 6.5 | Ph. Eur. 9.2 2.2.3 (potentiometry) |
| Water (% w/w) | 0.78 | 2.21 | 2.39 | 3.96 | 5.67 | 2.74 | Karl Fischer titration (coulometric titration) |
| Ash, sulphated (% w/w) | 0.08 | < 0.01 | < 0.01 | < 0.01 | 0.10 | < 0.01 | Ph. Eur. 9.2 2.4.14 (gravimetry) |
| Protein (% w/w) | < 0.0017 | < 0.0017 | 0.0091 | < 0.0017 | < 0.0017 | < 0.0017 | Bradford assay (spectrophotometry) |
| Contaminants | | | | | | | |
| Arsenic (total) (mg/kg) | < 0.1 | < 0.1 | < 0.1 | 0.1 | < 0.1 | < 0.1 | MSZ EN 13805:2015, EPA 6020A:2007 (ICP-MS) |
| Cadmium (mg/kg) | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 | DIN EN 15763:2010 (2010–04), mod. (ICP-MS) |
| Lead (mg/kg) | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 | |
| Mercury (mg/kg) | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 | |
| Aflatoxin M1 (µg/kg) | < 0.020 | < 0.020 | < 0.020 | < 0.020 | – | – | LC–MS/MS (internal method) |
| Microbial parameters | | | | | | | |
| Total plate count (CFU/g) | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | ISO 4833-1 or ISO-4833-2 MSZ ISO 15213:2006 (colony count) |
| Yeasts and moulds (CFU/g) | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | MSZ ISO 21527-2:2013 (colony count) |
| Enterobacteriaceae (in 10 g) | 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 | AFNOR BRD 07/11–12/05 MSZ EN ISO 6579-1:2017 (detection or qualitative method) |
| *Cronobacter* spp. (in 10 g) | ND | ND | ND | ND | ND | ND | MSZ EN ISO 22964:2017 (detection or qualitative method) |
| *Listeria monocytogenes* (in 25 g) | ND | ND | ND | ND | ND | ND | MSZ EN ISO 11290-1:2017 (detection or qualitative method) |
| Presumptive *Bacillus cereus* (CFU/g) | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | MSZ EN ISO 7932:2005 (colony count) |
| Endotoxins (EU/mg) | 0.1398 | 0.0023 | 0.0357 | 0.0107 | < 0.00025 | 0.0012 | Ph. Eur. 2.6.14 (LAL kinetic chromogenic assay) |

Abbreviations: 2′-FL: 2′-Fucosyllactose; 3-FL: 3-Fucosyllactose; AFNOR: Association Francaise de Normalisation; BRD: Bacteriology Reference Department; CFU: Colony forming units; DIN: Deutsches Institut für Normung e.V.; DM: Dry matter; EN: European norm; EPA: Environmental Protection Agency; EU: endotoxin units; HPAEC–PAD: High-performance anion-exchange chromatography – pulsed amperometric detection; HPLC–CAD: High-performance liquid chromatography – charged aerosol detection; ICP-MS: Inductively coupled plasma – mass spectrometry; ISO: International Organisation for Standardisation; LAL: Limulus amebocyte lysate; LC–MS/MS: Liquid chromatography – tandem mass spectrometry; m/mod.: Modification of analytical methods; MSZ: Hungarian Standards Institution; ND: Not detected; Ph. Eur.: European Pharmacopoeia; w/w: Weight per weight.

[a]Specified saccharides include LNFP-I, 2′-FL, LNT, DFL, 3-FL, D-lactose, sum of L-fucose and 2′-fucosyl-D-lactitol, LNFP-I fructose isomer and 2′-fucosyl-D-lactulose.

[b]L-Fucose and 2′-fucosyl-lactitol peaks on the HPAEC–PAD chromatogram overlap.

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 5-year (real time) stability study at 25°C and 60% relative humidity (RH) with one batch of the NF. The applicant also carried out a 2-year stability study under accelerated conditions (40°C, 75% RH) with the same batch of the NF. Results up to 24 months were provided for both stability studies, including sensory parameters, and carbohydrate and water content. Microbial parameters were also monitored up to 12 and 24 months under normal and accelerated storage conditions, respectively. No appreciable changes in the organoleptic properties, carbohydrate, LNFP-I, 2′-FL and moisture content were observed up to 24 months of storage under normal and accelerated conditions. Microbial parameters were also below the respective limits of detection over the 12-month and 24-month storage period under normal and accelerated conditions, respectively.

Moreover, the applicant provided the results of a 3-year (real time) stability study at (non-controlled) ambient temperature and RH conditions with two batches of the NF, with no appreciable changes for the above-mentioned parameters.

The applicant also provided the results of different stressed/forced stability studies with a batch of the NF in solid state or aqueous solution, indicating as follows:

- No appreciable variability in the LNFP-I and 2′-FL content was observed when the NF in powdered solid state was stored at 80°C for 30 days at two different levels of air humidity.
- Two potential pH-dependent degradation pathways were proposed as a result of the tests carried out in aqueous solution (10 mg/mL) at 60°C and different pH conditions (unbuffered or buffered at pH 5.0 or 6.8 for 28 days; buffered at pH 3.0 or 9.0 for 7 days; in 0.1 N HCl or 0.01 N NaOH for 1 day):
    - Under acidic conditions (pH < 3), hydrolysis of LNFP-I into several degradation products may occur, mainly LNT and fucose, but also glucose, lactose, 2-fucosyl-galactose, lacto-N-triose II, 2′-fucosyl-lacto-N-biose, 2″-fucosyl-lacto-N-triose I, LNFP-I fructose isomer, 3-Gal-lactose, 6-α-Gluf-Glu and other compounds.
    - Under basic conditions, a possible degradation pathway starts with the isomerisation of LNFP-I to the fructose isomer already at pH = 6.8 and continues with the so-called pealing reaction as pH increases. Typical degradation products include LNFP-I fructose isomer, 2-fucosyl-galactose, 2″-fucosyl-lacto-N-triose I, anhydro-GlcNAc I and an unknown compound at HPAEC retention time 21.7 min. In addition, fucose, galactose, glucose, 2-fucosyl-lactulose and other compounds may occur. However, the content of lacto-N-triose II and LNT may not change significantly.
- No degradation products were identified in the study conducted in aqueous solution (10 mg/mL) at room temperature for 1 day in presence of the oxidising agent 4,4′-azobis-(4-cyanovaleric acid) (ACVA) in 1:0.1 molar ratio. In 0.1% hydrogen peroxide, under the same storage conditions, the content of glucose, lactose, lacto-N-triose and some compounds slightly increased, and a significant peak for an unknown compound at HPAEC retention time 7.6 min was observed at the beginning of the study and remained stable over the stability period.

The applicant proposed a 5-year shelf-life under ambient conditions for the NF.

The Panel considers that the available data provided sufficient information with respect to the stability of the NF for 36 months.

**Stability of the NF under the intended conditions of use**

A 3-year stability study (ongoing) was conducted with powdered IF produced under representative conditions for commercial products. The IF was supplemented with the NF (1% w/w DM LNFP-I/2′-FL) and stored at 5°C, 25°C/60% RH, 30°C/65% RH or 40°C/75% RH. Interim results, including LNFP-I and 2′-FL content and microbial parameters, showed that LNFP-I and 2′-FL are stable up to 12 months under the above-mentioned storage conditions.

Additional studies demonstrated that LNFP-I and 2′-FL are stable in formulations representative of commercial food products on the market under typical processing and storage conditions for such products, as follows: cereal bars subject or not to a heating step over 100°C (up to 3 months at ambient conditions); pasteurised juice drink (up to 28 days at 5°C), pasteurised ready-to-drink milkshake (up to 14 days at 5°C), UHT ready-to-drink milkshake (up to 28 days at 5°C) and fruit yoghurt (up to 21 days at 5°C).

In addition, the stability of the authorised 2′-FL has been demonstrated in various food matrices, including IF, whole/UHT milk, yoghurt, ready-to-drink flavoured milk, citrus fruit beverages and cereal bars (EFSA NDA Panel, 2015a; EFSA NDA Panel, 2019a, 2022a).

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:** LNFP-I/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* K-12 DH1 MDO MP2173b) of *E. coli* K-12 DH1 (DSM 4235) | |
| **Parameter** | **Specification** |
| Composition | |
| Specified saccharides[a] (% w/w DM) | ≥ 90.0 |
| LNFP-I and 2′-FL (% w/w DM) | 75.0–100.0 |
| LNFP-I (% w/w DM) | 50.0–75.0 |
| 2′-FL (% w/w DM) | 15.0–35.0 |
| LNT (% w/w) | ≤ 5.0 |
| 3-FL (% w/w) | ≤ 1.0 |
| Sum of L-fucose and 2′-fucosyl-lactitol[b] (% w/w) | ≤ 1.0 |
| D-Lactose (% w/w) | ≤ 10.0 |
| DFL (% w/w) | ≤ 2.0 |
| LNFP-I fructose isomer (% w/w) | ≤ 1.5 |
| 2′-fucosyl-D-lactulose (% w/w) | ≤ 1.0 |
| Sum of other carbohydrates | ≤ 6.0 |
| pH (5% solution, 20°C) | 4.0–7.0 |
| Water (% w/w) | ≤ 8.0 |
| Ash (% w/w) | ≤ 0.5 |
| Protein (% w/w) | ≤ 0.01 |
| Contaminants | |
| Arsenic (mg/kg) | ≤ 0.2 |
| Cadmium (mg/kg) | ≤ 0.1 |
| Lead (mg/kg) | ≤ 0.02 |
| Mercury (mg/kg) | ≤ 0.1 |
| Aflatoxin M1 (μg/kg) | ≤ 0.025 |
| Microbial 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 |

Abbreviations: 2′-FL: 2′-Fucosyllactose; 3-FL: 3-Fucosyllactose; CFU: Colony forming units; DFL: Difucosyllactose; EU: Endotoxin units; LNFP-I: Lacto-N-fucopentaose I; LNT: Lacto-N-tetraose; ND: Not detected.

[a]Specified saccharides include LNFP-I, 2′-FL, LNT, DFL, 3-FL, D-lactose, L-fucose and 2′-fucosyl-lactitol, LNFP-I fructose isomer and 2′-fucosyl-D-lactulose.

[b]L-Fucose and 2′-fucosyl-lactitol peaks on the HPAEC–PAD chromatogram overlap.

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.

LNFP-I, the major constituent of the NF, is a fucosylated derivative of LNT. LNT, produced by the same applicant via fermentation by genetically modified strains of *E. coli* K-12 DH1 or BL21 (DE3), and its constitutional isomer, LNnT, produced

by the same applicant via chemical synthesis and fermentation by genetically modified strains of *E. coli* K-12 DH1 or BL21 (DE3), have been included in the Union list of NF (see Section 1.2).

2′-FL, the other main 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).

### 3.6.2 | Intake of oligosaccharides constituent of the NF from human milk

As reported in previous EFSA opinions (EFSA NDA Panel, 2019b, 2020a, 2020b, 2021, 2022c), human milk contains a family of structurally related oligosaccharides, known as HMOs, which is the third largest fraction of solid components. The highest concentrations of HMOs occur in human colostrum (20–25 g/L), and concentrations between 5 and 20 g/L occur in mature human milk (Bode, 2012; Gidrewicz & Fenton, 2014; Thurl et al., 2010; Urashima et al., 2018). HMOs' concentrations and composition vary across mothers and over the course of lactation. 2′-FL and LNFP-I are both α1,2 fucosylated neutral oligosaccharides and are among the five most abundant HMOs (Molnar-Gabor et al., 2019; Thurl et al., 2017). The fraction of neutral fucosylated HMOs characterised by the presence of L-fucose accounts for up to 80% of the total HMO concentration (Bode, 2012; Rijnierse et al., 2011; Thurl et al., 2010).

Several publications on LNFP-I and 2′-FL in human milk have been provided by the applicant. In consideration of the large and recent data set used in this review (Soyyılmaz et al., 2021), and aligned with the recent EFSA opinions (e.g. EFSA NDA Panel, 2023b, 2023c), the Panel decided to use the values reported there for the mean of mean concentrations and the maximum mean concentration as representative of the concentration range found in mature human milk.

For LNFP-I, these values correspond to 0.83 g/L and 2.14 g/L, respectively. The Panel also notes that due to the relatively wide concentration range of LNFP-I in human milk (up to 3.03 g/L – Austin et al., 2019; 3.76 g/L Samuel et al., 2019), higher natural intakes may occur.

For 2′-FL, that is the most represented oligosaccharide in human milk, these values correspond to 2.28 g/L and 4.28 g/L, respectively. 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 the mean of mean concentrations and the maximum mean concentration as representative of the range found in mature human milk and considering the average and high daily intakes of human milk (800 and 1200 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 (LNFP-I) and Table 5 (2′-FL).

**TABLE 4** Estimated daily intakes of LNFP-I from average (800 mL) and high (1200 mL) daily intakes of human milk for infants of 6.7 kg body weight (bw), based on the mean of mean concentrations (0.83 g/L) and the maximum mean concentration (2.14 g/L) of LNFP-I in mature human milk (lactation days 15–90; Soyyılmaz et al., 2021).

| | Daily intake of LNFP-I (mg/kg bw) from 800 mL/day of human milk | | Daily intake of LNFP-I (mg/kg bw) from 1200 mL/day of human milk | |
|---|---|---|---|---|
| | Mean of mean concentrations | Maximum mean concentration | Mean of mean concentrations | Maximum mean concentration |
| LNFP-I | 99 | 256 | 149 | **383** |

Abbreviation: bw, body weight.

**TABLE 5** Estimated daily intakes of 2′-FL from average (800 mL) and high (1200 mL) daily intakes of human milk for infants of 6.7 kg body weight (bw), based on the mean of mean concentrations (2.28 g/L) and the maximum mean concentration (4.28 g/L) 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 1200 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** |

Abbreviation: bw, body weight.

In bovine milk, oligosaccharides are 20 times less concentrated than in human milk and acidic oligosaccharides are the most abundant oligosaccharides (e.g. 6′-SL), while fucosylated ones (e.g. LNFP-I, 2′-FL) are found at 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 NF is proposed to be used as an ingredient in various food categories, including IF and FOF. These food products, defined using the FoodEx2 hierarchy, and the proposed maximum use levels, are reported in Table 6.

The applicant also intends to market the NF for use in FS as defined in Directive 2002/46/EC. Specifically, maximum daily intakes of 4.5 g/day for individuals of 3 years of age and above, or 2.25 g/day when intended for infants and young children have been proposed.

For FSMP, 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 FS (see Section 3.7.4).

FS are not intended to be used if other foods with added NF or human milk (in infants and young children) are consumed on the same day.

**TABLE 6** Food categories according to FoodEx2 hierarchy and maximum use levels of the NF intended by the applicant.

| FoodEx2 code | FoodEx2 level | Food category | Proposed max. use levels (mg LNFP-I/2′-FL/100 g) |
|---|---|---|---|
| A02LV | 5 | Cow milk | 150 |
| A0CXA | 5 | European buffalo milk | 150 |
| A02MC | 5 | Sheep milk | 150 |
| A02MB | 4 | Goat milk | 150 |
| A02MV | 3 | Butter milk | 150 |
| A02NQ | 4 | Yoghurt drinks, including sweetened and/or flavoured variants | 150 |
| A02NR | 4 | Probiotic milk-like drinks | 150 |
| A02NV | 5 | Kefir | 150 |
| A02NE | 4 | Yoghurt | 300 |
| A00EY | 3 | Cereal bars | 1500 |
| A03PZ | 4 | Infant formulae, powder | 1600[a] |
| A03QE | 4 | Infant formulae, liquid | 200[a] |
| A03QK | 4 | Follow-on formulae, powder | 1600[a] |
| A0EQQ | 4 | Follow-on formulae, liquid | 200[a] |
| A03QZ | 3 | Cereals with an added high protein food which have to be reconstituted | 600 |
| A03QY | 3 | Simple cereals which have to be reconstituted | 1050 |
| A0BZF | 3 | Cereals with added high protein food reconstituted | 150 |
| A0BZE | 3 | Simple cereals for infants and children reconstituted | 150 |
| A03RA | 3 | Biscuits, rusks and cookies for children | 910 |
| A03RC | 2 | Ready-to-eat meal for infants and young children | 910 |
| A03RB | 3 | Pasta for children (dry, to be cooked) | 910 |
| A03RN | 3 | Fruit and vegetable juices and nectars specific for infants and young children | 150 |
| A0EQN | 5 | Soft drinks with minor amounts of fruits or flavours | 150 |
| A03RP | 3 | Special food for children's growth | 910 |

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

### 3.7.3 | Anticipated intake of the NF

**Anticipated intake of LNFP-I/2′-FL mixture from the consumption of the NF in IF in infants up to 16 weeks of age**

IF is expected to be the only food consumed by infants aged 0–16 weeks who are not breastfed. A high consumption of IF has been estimated to be 260 mL/kg bw per day for infants aged 0–16 weeks (EFSA Scientific Committee, 2017). Based on the maximum proposed use level of the NF (2.0 g/L in IF), the high intake of the NF from IF alone is estimated to be 520 mg/kg bw per day, corresponding to about 390 mg LNFP-I/kg bw and 182 mg 2′-FL/kg bw (assuming 75% and 35% of the NF as per upper limits of the proposed ranges in specifications, respectively).

The Panel notes that the highest anticipated daily intake of the NF from the consumption of IF (only) may result in levels that are similar to the estimated highest natural mean daily intake for LNFP-I (383 mg/kg bw per day; Table 4) in breastfed infants. For 2′-FL the highest intake values are lower than highest natural mean intake (767 mg/kg bw per day; Table 5).

**Anticipated intake of LNFP-I/2′-FL mixture 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 6), using the EFSA Dietary Exposure (DietEx) Tool,[16] 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 intake of the NF (expressed as LNFP-I/2′-FL mixture on a mg/kg bw basis), among the EU dietary surveys, are presented in Table 7.

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 the Supporting Information section.

**TABLE 7** Intake estimate of LNFP-I/2′-FL mixture resulting from the use of the NF as an ingredient in the intended food categories at the maximum proposed use levels.

| | | Mean intake (mg/kg bw per day) | | P95 intake (mg/kg bw per day) | |
|---|---|---|---|---|---|
| **Population group** | **Age (years)** | **Lowest[a]** | **Highest[a]** | **Lowest[b]** | **Highest[b]** |
| Infants | < 1 | 71 | 293 | 189 | 588 |
| Young children[c] | 1 to < 3 | 37 | 155 | 103 | 555 |
| Other children | 3 to < 10 | 14 | 49 | 31 | 85 |
| Adolescents | 10 to < 18 | 3 | 19 | 12 | 44 |
| Adults[d] | ≥ 18 | 8 | 10 | 17 | 24 |

Abbreviation: bw, body weight.

[a]Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 27 June 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 27 June 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.

Considering the upper limits of the range included in the specifications, 75% of the NF for LNFP-I and 35% for 2′-FL, the highest P95 intake calculated for LNFP-I are 441 and 416 mg/kg bw per day in infants and young children, respectively (Table 8). The Panel notes that both results are higher than the estimated natural highest mean daily intake of 383 mg/kg bw per day (Table 4).

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

For the other population groups the intake is lower (18–54 mg/kg bw per day; Table 8). The panel finally notes that the calculated high intakes of 2′-FL in all population groups (8–206 mg/kg bw per day; Table 9), are below the estimated natural highest mean daily intake of 767 mg/kg bw per day (Table 5).

**TABLE 8** Intake estimate of LNFP-I (upper limit of the proposed range in specifications) resulting from the use of the NF as an ingredient in the intended food categories at the maximum proposed use levels.

| | | LNFP-I mean intake (mg/kg bw per day)[e] | | LNFP-I P95 intake (mg/kg bw per day)[e] | |
|---|---|---|---|---|---|
| **Population group** | **Age (years)** | **Lowest[a]** | **Highest[a]** | **Lowest[b]** | **Highest[b]** |
| Infants | < 1 | 53 | 220 | 142 | 441 |
| Young children[c] | 1 to < 3 | 28 | 116 | 77 | 416 |
| Other children | 3 to < 10 | 11 | 37 | 23 | 54 |
| Adolescents | 10 to < 18 | 2 | 14 | 9 | 33 |
| Adults[d] | ≥ 18 | 6 | 8 | 13 | 18 |

Abbreviation: bw, body weight.

[a]Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 27 June 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 27 June 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.

[e]Assuming for LNFP-I 75% of the NF as per upper limit in the specifications.

**TABLE 9** Intake estimate of 2′-FL (upper limit of the proposed range in specifications) resulting from the use of the NF as an ingredient in the intended food categories at the maximum proposed use levels.

| | | 2′-FL mean intake (mg/kg bw per day)[e] | | 2′-FL P95 intake (mg/kg bw per day)[e] | |
|---|---|---|---|---|---|
| **Population group** | **Age (years)** | **Lowest[a]** | **Highest[a]** | **Lowest[b]** | **Highest[b]** |
| Infants | < 1 | 25 | 103 | 66 | 206 |
| Young children[c] | 1 to < 3 | 13 | 54 | 36 | 194 |
| Other children | 3 to < 10 | 5 | 17 | 11 | 30 |
| Adolescents | 10 to < 18 | 1 | 7 | 4 | 15 |
| Adults[d] | ≥ 18 | 3 | 4 | 6 | 8 |

Abbreviation: bw, body weight.

[a]Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 27 June 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 27 June 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.

[e]Assuming for 2′-FL 35% of the NF as per upper limit in the specifications.

### 3.7.4 | Anticipated intake of LNFP-I/2′-FL mixture from the use as FS

The applicant has proposed a maximum daily intake of 4.5 g LNFP-I/2′-FL mixture/day as FS for individuals 3 years and above of age and a maximum level of 2.25 g NF/day for infants (0–11 months) and young children (12–35 months).

**TABLE 10** Intake estimate of LNFP-I/2′-FL mixture resulting from the use of the NF in FS.

| Population group | Age (years) | Body weight[a] (kg) | Use level LNFP-I/2′-FL (g/day) | Use level LNFP-I/2′-FL (mg/kg bw per day)[b] | Intake of LNFP-I (mg/kg bw per day)[e] | Intake of 2′-FL (mg/kg bw per day)[e] |
|---|---|---|---|---|---|---|
| Infants | < 1 | 5.0 | 2.25 | 450 | 338 | 158 |
| Young children[c] | 1 to < 3 | 12.0 | 2.25 | 188 | 141 | 66 |
| Other children | 3 to < 10 | 23.1 | 4.5 | 195 | 146 | 68 |
| Young adolescents | 10 to < 14 | 43.4 | 4.5 | 104 | 78 | 36 |
| Older adolescents | 14 to < 18 | 61.3 | 4.5 | 73 | 55 | 26 |
| Adults[d] | ≥ 18 | 70.0 | 4.5 | 64 | 48 | 22 |

Abbreviation: bw, body weight.

[a]Default and average body weights for each population group are available in EFSA Scientific committee (2012).

[b]: Intake in 'mg/kg bw per d' are calculated by considering the use levels in 'mg/d' and default body weights defined in EFSA Scientific Committee (2012).

[c]Referred as 'toddlers' in the EFSA food consumption comprehensive database (EFSA, 2011).

[d]Includes elderly, very elderly, pregnant and lactating women.

[e]Assuming 75% and 35% of the NF for LNFP-I and 2′-FL, respectively, as per upper limits in the specifications.

The intake of LNFP-1 from the use of the NF as FS (Table 10) shows that the maximum daily intake (i.e. 48–338 mg/kg bw per day) is lower than the estimated natural highest mean daily intake of LNFP-I of 383 mg/kg bw in breastfed infants (Table 4). Similarly, the maximum daily intake of 2′-FL from the use of the NF as FS (i.e. 22–158 mg/kg bw per day) is below the high intake of 2′-FL of 767 mg/kg bw in breastfed infants (Table 5).

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

Finally, the applicant proposed the use of the NF in 'Total daily diet replacement for weight reduction' at a maximum daily intake of 4.5 g LNFP-I/2′-FL mixture. The use is limited to 'healthy overweight or obese adults'[10] and the resulting intake is lower than the estimated highest mean daily intake in breastfed infants on a body weight basis (Table 10).

### 3.7.5 | Combined intake from the NF and other sources

The Panel notes that the main component of the NF, LNFP-I, is not authorised for use in food categories other than those proposed for the NF under assessment. Therefore, the only possible additional source for LNFP-I is human milk.

2′-FL is already authorised for use in several food categories.[4] The Panel notes that the food categories where 2′-FL is proposed to be added are similar to the authorised ones. However, since the use is authorised in a few other food categories (e.g. table-top sweeteners) a combined intake of the current LNFP-I/2′-FL mixture with foods containing 2′-FL only may occur. The Panel also notes that the intake with the current proposed conditions of use as NF is rather low in comparison with the estimated highest natural daily intake and the already authorised uses and use levels.

## 3.8 | Absorption, distribution, metabolism and excretion (ADME)

No ADME data were provided for the NF.

As mentioned by the applicant and reported in previous EFSA opinions (e.g. EFSA NDA Panel, 2015a; EFSA NDA Panel, 2022c, 2023a) 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 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 LNFP-I and 2′-FL, or other structurally related mono- and oligosaccharides (e.g. D-lactose, LNT) from the NF, differs from that of the same components in human milk.

## 3.9 | Nutritional information

The NF is mainly composed of the non-digestible oligosaccharides LNFP-I and 2′-FL.

The NF contains other carbohydrates individually present at low concentrations (slightly above or below 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 and 3-FL also belong to the group of fucosylated HMOs, which constitute up to 80% of the total HMO fraction in human milk (Bode, 2012). LNT

is also one of the most relevant HMOs present in all types of human milks (Erney et al., 2001). Only traces of other related oligosaccharides (e.g. 2′-fucosyl-D-lactulose) can be detected in the NF.

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 three toxicological studies on the NF, which were conducted in compliance with Organisation for Economic Co-operation and Development (OECD, 1997, 2014, 2018) principles of Good Laboratory Practice (GLP) (OECD, 1998) and in accordance with relevant OECD test guidelines (TG) No 471, 487 and 408. The studies were conducted with the same batch of the NF which contained 89.7% w/w of LNFP-I/2′-FL (about 59% and 32%, respectively). The studies which were claimed proprietary by the applicant are listed in Table 11. Experimental designs with main results are included in a relevant publication (Phipps et al., 2020).

**TABLE 11** List of toxicological studies with the NF provided by the applicant.

| Reference | Type of study | Test system | Dose (LNFP-I/2′-FL) |
|---|---|---|---|
| Study No. YP48JX, technical report 2020a (Phipps et al., 2020) | Bacterial reverse mutation test (GLP, OECD TG 471 (1997)) | *Salmonella* Typhimurium TA98, TA100, TA1535 and TA1537. *Escherichia coli* WP2 uvrA (pKM101) | Up to 5000 μg/plate (absence and presence of S9 mix) |
| Study No. QR38KG, technical report 2020b (Phipps et al., 2020) | *In vitro* mammalian cell micronucleus test (GLP, OECD TG 487 (2014)) | Human lymphocytes | 500, 1000 and 2000 μg/mL (absence and presence of S9 mix) |
| Study No. FC89HQ, technical report 2020c (Phipps et al., 2020) | 90-day repeated dose oral toxicity study followed by a 4-week recovery period (GLP, OECD TG 408 (2018)) | Neonatal Crl:CD(SD) rats | 0, 1000, 3000 or 5000 mg/kg bw/day (oral gavage from day 7 of age) |

Abbreviations: bw, body weight; GLP, Good Laboratory Practice; OECD, Organisation for Economic Co-operation and Development; SD, Sprague Dawley; TG, test guidelines.

### 3.10.1 | Genotoxicity

The potential genotoxicity of the NF was investigated in a bacterial reverse mutation test and in an in vitro mammalian cell micronucleus test (Table 11).

The in vitro assessment of the mutagenic potential of the NF (Study Report, 2020a; Phipps et al., 2020) 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 55.5 up to 5550 μg NF/plate (corresponding to 5000 μg LNFP-I/2′-FL mixture) in the 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 two-fold increase) over control counts were observed with any of the strains following exposure to the LNFP-I/2′-FL mixture at any concentration. No appreciable toxicity or precipitation was observed following exposure to any tested dose of the NF.

In the in vitro mammalian cell micronucleus test in human lymphocytes (Phipps et al., 2020; Study Report, 2020b), concentrations of 555, 1110 and 2220 μg NF/mL (corresponding to up to 2000 μg/mL of LNFP-I/2′-FL mixture) were tested in the main study in the presence (3-hour treatment) and absence (3- and 20-hour treatments) of S9 metabolic activation. No cytotoxicity or precipitation were observed and the percentage of micronuclei in cultured human lymphocytes was not significantly increased in any of the test substance concentrations.

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) neonatal rats/sex were administered by gavage a dose of 0 (vehicle, water), 1110, 3330 and 5550 mg NF/kg bw per day (corresponding to 1000, 3000 or 5000 mg/kg bw of LNFP-I/2′-FL mixture) once daily for 90 consecutive days, starting from 7-day of age. An additional reference control group received oligofructose at 5000 mg/kg bw per day under the same conditions. Additional five rats/sex in the vehicle control, high-dose NF and reference control groups were also dosed once daily for 90 days and then observed over a 4-week recovery period, to assess the reversibility of any changes observed in the dosing phase (Phipps et al., 2020; Study Report, 2020c).

In addition to the standard examinations and data collection (including functional observational battery and some hormonal measurements) and in consideration of the age of the rats, specific observations were carried out, including

pre-weaning reflex development (e.g. eye opening, startle response), ulna length, sexual maturation (balano-preputial separation and vaginal opening for males and females, respectively) and oestrous cycle monitoring.

There were no test-item related deaths in the course of the study and no treatment-related clinical signs were observed in any rats. A total of nine rats died or were sacrificed because of poor general condition in the first 2-week of dosing, of which five were in the reference control group, two in the mid-dose group and two in the high-dose group. Apart from one rat in the high-dose group, whose death was due to a dosing error, no clear cause of death for the remaining ones was determined. No changes in body weight and food consumption through the study were noted and no biologically relevant differences in the age or body weight at which the males and females attained physical signs of sexual maturation were observed. The mean body weights at balano-preputial skinfold separation for mid- and high-dose males and also in the reference control males were statistically significantly higher than those of vehicle controls, without dose-correlation. Oestrous cycles were unaffected by the NF administration.

Statistically significant differences in some haematological parameters were noted: increased neutrophil count for males (mid- and high-dose, not dose-related), monocytes and large unstained cells for mid-dose males. Decreased platelet counts for all groups in both sexes, reference controls included, were also noted. Finally, decreased reticulocyte counts for low- and mid-dose females, decreased mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration for high-dose females, and shortened prothrombin time for all females were recorded. Likewise, in clinical biochemistry statistically significant changes were noted: increased inorganic phosphorus for low- and mid-dose females and decreased alanine aminotransferase in high-dose females. Slight increases in T3 levels were observed at high-dose in both sexes and in the reference control group. Slight increases in TSH for low- and mid-dose males, and for mid-dose females were also observed. In urinary parameters statistically significant increases in urinary pH (all male groups and mid- and high-dose females) and decreased specific gravity limited to high-dose males were noted.

The Panel notes that these changes (all reported in Phipps et al., 2020) were generally of low magnitude, without a clear dose–response, not consistently observed in both sexes and sometimes also noted in the reference control group. For the above reasons, the changes are overall considered by the Panel as not toxicologically relevant.

The statistically significant changes observed in organ weights were lower salivary gland weight in mid-dose males, reductions in adrenal weight (high-dose males) and brain, lungs and bronchi weight (high-dose females). Overall, there were no NF-related macroscopic or histological abnormalities. The findings observed were considered incidental, with low frequency, distributed across groups and generally consistent with changes observed in SD rats in subchronic studies.

The Panel considers that no adverse effects were observed in this study up to the highest tested dose of 5000 mg LNFP-I/2′-FL mixture /kg bw per day (5550 mg NF/kg bw per day).

### 3.10.3 | Human data

No human studies with the NF have been conducted according to the applicant.

## 3.11 | Allergenicity

The applicant did not identify an allergenic potential of introduced proteins as a result of the genetic modification of *E. coli* K-12 DH1 (DSM 4235) 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 LNFP-I and 2′-FL, but it also contains D-lactose, LNT, DFL, LNFP-I fructose isomer, 3-FL, 2′-fucosyl-D-lactulose, L-fucose and 2′-fucosyl-D-lactitol, and a small fraction of other related saccharides. The NF is produced by fermentation by a genetically modified strain (*E. coli* K-12 DH1 MDO MP2173b) of *E. coli* K-12 DH1 (DSM 4235).

The applicant intends to add the NF to a variety of foods (e.g. milk, yoghurt, cereals), including IF and FOF, FSMP and FS. The target population proposed by the applicant is the general population.

It is noted that additional sources of 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 3.6.2). Considering that LNFP-I is not included in the Union list of NF, it 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 to LNFP-I relates to breastfed infants. 2′-FL when differently produced is already authorised for use in several food categories. The Panel notes that in infants up to 16 weeks of age the anticipated daily intake of LNFP-I from the consumption of IF only, is

similar to the corresponding estimated highest mean daily intake in breastfed infants on a body weight basis. Values up to 15% higher than the estimated highest mean daily intake from breastmilk were noted in infants (in 2 out of 12 dietary surveys included in the EFSA food consumption database) and in young children (1 out of 15 surveys). Considering the conservative assumption underlying this type of intake assessment (in particular, assuming that the NF is added at the maximum proposed use levels, at the upper level of the range included in the specifications and to all the proposed food categories consumed by infants and young children), the Panel considers that it is unlikely that the intake of LNFP-I would exceed the estimated highest mean daily intake in breastfed infants. Estimated natural highest mean daily intakes were not exceeded in all conditions of use for 2′-FL, FS included.

According to the applicant, FS are not intended to be used if other foods to which LNFP-I and 2′-FL have been added (as well as human milk for infants and young children) are consumed on the same day.

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 containing LNFP-I and 2′-FL for the proposed uses at their respective maximum use levels can be considered safe.

The submitted toxicity studies did not raise safety concerns. No toxicologically relevant effects were observed in the subchronic toxicity study performed in neonatal SD rats at up to the highest dose tested of 5000 mg LNFP-I/2′-FL mixture/kg bw per day (5550 mg NF/kg bw per day).

It is finally noted that, in line with other oligosaccharides that are natural components of human milk, the safety assessment of the components of this NF is mainly based on the comparison between the natural intake in breastfed infants and the estimated intake of NF components. 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 a mixture of LNFP-I and 2′-FL in the NF under the proposed conditions of use does not raise safety concerns.

## 5 | CONCLUSIONS

The Panel concludes that the NF, which is composed of a mixture of LNFP-I/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–CAD; (ii) detailed description of the production process; (iii) information on the genetically modified production strain; (iv) composition and stability of the NF; (v) intake assessment; (vi) toxicological information, including *in vitro* genotoxicity studies and 90-day subchronic toxicity study (Table 11).

## 6 | STEPS TAKEN BY EFSA

1. On 01 July 2021 EFSA received a letter from the European Commission with the request for a scientific opinion on the safety of Lacto-N-fucopentaose I/2′-fucosyllactose mixture. Ref.Ares(2021)4282145.
2. On 01 July 2021, a valid application on the safety of Lacto-N-fucopentaose I/2′-fucosyllactose mixture, which was submitted by Glycom A/S, was made available to EFSA by the European Commission through the Commission e-submission portal (NF 2021/2371) and the scientific evaluation procedure was initiated.
3. On 15 October 2021, 20 May 2022, 05 January 2023 and 12 May 2023, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4. On 02 February 2022, 05 January 2023, 19 April 2023 and 07 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 October 2023, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of Lacto-N-fucopentaose I/2′-fucosyllactose mixture as a NF pursuant to Regulation (EU) 2015/2283.

### ABBREVIATIONS

| | |
|---|---|
| 1D | Mono-dimensional |
| 2D | Two-dimensional |
| 2′-FL | 2′-Fucosyllactose |
| 3-FL | 3-Fucosyllactose |
| 3′-SL | 3′-Sialyllactose |
| 6′-SL | 6′-Sialyllactose |
| ACVA | 4,4′-azobis-(4-cyanovaleric acid) |

| | |
|---|---|
| ADME | Absorption, Distribution, Metabolism and Excretion |
| AFNOR | Association Francaise de Normalisation |
| APC | Adenomatous polyposis coli |
| ATCC | American Type Culture Collection |
| BIOHAZ | EFSA Panel on Biological Hazards |
| BRD | Bacteriology Reference Department |
| bw | Body weight |
| CAS | Chemical Abstracts Service |
| CFU | Colony forming units |
| CID | Collision-induced dissociation |
| Crl:CD(SD) rats | Charles River Laboratories: Caesarean-derived (Sprague Dawley) rats |
| DEPT-Q | Distortionless enhancement by polarisation transfer with retention of quaternaries |
| DFL | Difucosyllactose |
| DietEx | EFSA Dietary Exposure tool |
| DIN | Deutsches Institut für Normung e.V. |
| DM | Dry matter |
| DSMZ | German Collection of Microorganisms and Cell Cultures |
| EC | European Commission |
| EFSA | European Food Safety Authority |
| EN | European norm |
| *Escherichia coli* W | Waksman's *E. coli* strain |
| EU | Endotoxin units |
| 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 22,000 |
| Gal | Galactose |
| g-DQFCOSY | Gradient double-quantum-filtered correlation spectroscopy |
| g-HSQC | Gradient heteronuclear single quantum coherence |
| g-HMBC | Gradient heteronuclear multiple bond coherence |
| Glc | Glucose |
| GlcNAc | N-acetyl-D-glucosamine |
| GLP | Good Laboratory Practices |
| GMO | EFSA Panel on Genetically Modified Organisms |
| GMP | Good Manufacturing Practices |
| HACCP | Hazard Analysis Critical Control Points |
| HiMO | Human-identical milk oligosaccharide |
| HMO | Human milk oligosaccharide |
| HPAEC–PAD | High-performance anion-exchange chromatography – pulsed amperometric detection |
| HPLC–CAD | High-performance liquid chromatography – charged aerosol detection |
| ICP-MS | Inductively coupled plasma – mass spectrometry |
| IF | Infant formula |
| ISO | International Organisation for Standardisation |
| IUPAC | International Union of Pure and Applied Chemistry |
| LAL | Limulus amebocyte lysate |
| LC | Liquid chromatography |
| LNFP-I | Lacto-N-fucopentaose I |
| LNnT | Lacto-N-neotetraose |
| LNT | Lacto-N-tetraose |
| LOQ | Limit of quantification |
| m/mod. | Modification of analytical methods |
| MDO | Membrane-derived oligosaccharides |
| MN | Micronucleous |
| MS/MS | Tandem mass spectrometry |
| MSZ | Hungarian Standards Institution |
| NANA | N-acetyl-D-neuraminic acid, sialic acid |
| ND | Not detected |
| NDA | EFSA Panel on Nutrition, Novel Foods and Food Allergens |
| NF | Novel food |
| NMR | Nuclear magnetic resonance spectroscopy |
| NOESY | Nuclear overhauser effect spectroscopy |

| | |
|---|---|
| OECD | Organisation for Economic Co-operation and Development |
| Ph. Eur. | European Pharmacopoeia |
| qPCR | Quantitative polymerase chain reaction |
| QPS | Qualified presumption of safety |
| RH | Relative humidity |
| RNA | Ribonucleic acid |
| SD | Standard deviation |
| SD rats | Sprague Dawley rats |
| T3 | Triiodothyronine |
| TG | Test guidelines |
| TOCSY | Total correlation spectroscopy |
| TSH | Thyroid stimulating hormone |
| UHT | Ultra-high temperature |
| US | United States |
| US EPA | US Environmental Protection Agency |
| w/w | Weight per weight |
| ZKBS | Central Committee on Biological Safety |

## CONFLICT OF INTEREST

If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

## REQUESTOR

European Commission

## QUESTION NUMBER

EFSA-Q-2021-00170

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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## APPENDIX 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. Annex A – can be found in the online version of this output (in the 'Supporting information' section).