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SCIENTIFIC OPINION

Safety of 3′-sialyllactose (3′-SL) sodium salt produced with a derivative strain (*Escherichia coli* K-12 MG1655 INB-3SL_01) of *E. coli* K-12 MG1655 (ATCC 700926) as a novel food pursuant to Regulation (EU) 2015/2283

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The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts.

Abstract

Following a request from the European Commission, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver an opinion on 3′-sialyllactose (3′-SL) sodium salt as a novel food (NF) pursuant to Regulation (EU) 2015/2283. The NF is mainly composed of the human-identical milk oligosaccharide (HiMO) 3′-SL (sodium salt), but it also contains D-lactose, sialic acid, 3′-sialyllactulose and a small fraction of other related saccharides. The NF is produced by fermentation with a genetically modified strain (*Escherichia coli* K-12 MG1655 INB-3SL_01) of *E. coli* K-12 MG1655 (ATCC 700926). 3′-SL (sodium salt) produced by fermentation with genetically modified strains of *E. coli* K-12 DH1, *E. coli* BL21 (DE3) or *E. coli* W (ATCC 9637) is already authorised and included in the EU list of NFs. This application refers to a change in the production process and specifications, while the target population, conditions of use and consequently the anticipated intake remain unchanged. The information provided on the identity, production process, composition and specifications and the toxicological data of the NF do not raise safety concerns. The intake of other carbohydrate-type compounds structurally related to 3′-SL is also considered of no safety concern. In line with other HiMOs, the safety assessment of this NF is mainly based on the comparison between the intake by breastfed infants and the estimated intake of the NF. Given that the NF would be consumed to the same extent as the already authorised 3′-SL (sodium salt), the Panel considers that the consumption of the NF at the proposed uses and use levels does not raise safety concerns. The Panel concludes that the NF is safe under the proposed conditions of use

KEYWORDS

3′-SL, 3′-sialyllactose, HiMO, HMO, human milk oligosaccharide, novel food, safety

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1 | INTRODUCTION

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

On 23 June 2023, the applicant "Inbiose N.V." submitted a request to the European Commission (EC) in accordance with Article 10 of Regulation (EU) 2015/2283[1] to authorise the placing on the market of 3′-sialyllactose (3′-SL) sodium salt produced with a derivative strain of *Escherichia coli* K12 as a novel food (NF).

The applicant requests to authorise the use of 3′-SL sodium salt produced with a derivative strain of *E. coli* K12 MG1655 in all the uses authorised by Commission Implementing Regulation (EC) 2021/96[2] for 3′-SL sodium salt following a NF application by another applicant.

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 EC asks the European Food Safety Authority (EFSA) to provide a scientific opinion on the safety of 3′-SL sodium salt produced with a derivative strain of *E. coli* K12 MG1655 as a NF.

In this opinion on 3′-SL sodium salt produced with a derivative strain of *E. coli* K12 MG1655 as a NF, 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

The sodium salts of 3′-SL and 6′-sialyllactose (6′-SL), a constitutional isomer of 3′-SL, are included in the Union list of authorised NFs (Commission Implementing Regulation (EU) 2017/2470[3]) when produced by fermentation with genetically modified strains of *Escherichia coli* K-12 DH1, *E. coli* BL21 (DE3) or *E. coli* W (ATCC 9637) (EFSA NDA Panel, 2020a, 2020b, 2022a, 2022b, 2023a, 2023b, 2026).

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′-fucosyllactose (2′-FL) (EFSA NDA Panel, 2015a) and 2′-FL produced with genetically modified strains of *Corynebacterium glutamicum* ATCC 13032 (EFSA NDA Panel, 2022c) or *E. coli* W (ATCC 9637) (EFSA NDA Panel, 2023c);
- Chemically synthesised lacto-N-neotetraose (LNnT) (EFSA NDA Panel, 2015b) and LNnT produced with genetically modified strains of *E. coli* BL21 (DE3) (EFSA NDA Panel, 2020c);
- Extension of use in food supplements (FS) for children of chemically synthetised 2′-FL and LNnT (EFSA NDA Panel, 2015c); extension of use in FS for infants of 2′-FL and LNnT produced with genetically modified strains of *E. coli* K-12 DH1 (EFSA NDA Panel, 2022d); and extension of use in infant formula (IF) and follow-on formula (FOF) of 2′-FL produced with a genetically modified strain of *E. coli* BL21 (DE3) (EFSA NDA Panel, 2023d);
- Chemically synthetised N-acetyl-d-neuraminic acid (NANA) (EFSA NDA Panel, 2017);
- 2′-FL/difucosyllactose (DFL) mixture produced with a genetically modified strain of *E. coli* K-12 DH1 (EFSA NDA Panel, 2019a);
- Lacto-N-tetraose (LNT) produced with genetically modified strains of *E. coli* K-12 DH1 (EFSA NDA Panel, 2019b), *E. coli* BL21 (DE3) (EFSA NDA Panel, 2022e) or *E. coli* K-12 MG1655 (EFSA NDA Panel, 2025a);
- Extension of use in FS for infants of 2′-FL/DFL mixture and LNT produced with genetically modified strains of *E. coli* K-12 DH1 (EFSA NDA Panel, 2022f);
- 3-fucosyllactose (3-FL) produced with genetically modified strains of *E. coli* K-12 MG1655 (EFSA NDA Panel, 2021a), *E. coli* BL21 (DE3) (EFSA NDA Panel, 2022g) or *E. coli* K-12 DH1 (EFSA NDA Panel, 2023e); and extension of use in IF, FOF, FS and food for special medical purposes (FSMP) of 3-FL produced with a genetically modified strain of *E. coli* BL21 (DE3) (EFSA NDA Panel, 2025b);
- 3′-sialyllactose (3′-SL) sodium salts produced with genetically modified strains of *E. coli* K-12 DH1 (EFSA NDA Panel, 2020a), *E. coli* BL21 (DE3) (EFSA NDA Panel, 2022a) or *E. coli* W (ATCC 9637) (EFSA NDA Panel, 2023a);
- 6′-sialyllactose (6′-SL) sodium salts produced with genetically modified strains of *E. coli* K-12 DH1 (EFSA NDA Panel, 2020b), *E. coli* BL21 (DE3) (EFSA NDA Panel, 2022b), *E. coli* W (ATCC 9637) (EFSA NDA Panel, 2023b) or *E. coli* K-12 MG1655 (EFSA NDA Panel, 2026);
- Lacto-N-fucopentaose I/2′-FL (LNFP-I/2′-FL) mixture produced with a genetically modified strain of *E. coli* K-12 DH1 (EFSA NDA Panel, 2023f);
- Scientific and technical assistance report on the evaluation of HiMOs as NFs (EFSA, 2024).

[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) 2021/96 of 28 January 2021 authorising the placing on the market of 3′-sialyllactose sodium salt as a novel food under Regulation (EU) 2015/2283 of the European Parliament and of the Council and amending Commission Implementing Regulation (EU) 2017/2470 (OJ L 31, 29.1.2021, p. 201).

[3]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.

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 EFSA requests 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.[4]

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, 2021b). 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.

The applicant has submitted a confidential and a non-confidential version of a dossier following the 'EFSA guidelines on the preparation and presentation of a NF application' (EFSA NDA Panel, 2021b) and the 'Administrative guidance for the preparation of applications on novel foods pursuant to Article 10 of Regulation (EU) 2015/2283' (EFSA, 2021).

In accordance with Art. 38 of Regulation (EC) No 178/2002[5] and taking into account the protection of confidential information and of personal data in accordance with Articles 39 to 39e of the same Regulation, and of the Decision of EFSA's Executive Director laying down practical arrangements concerning transparency and confidentiality,[6] the non-confidential version of the dossier has been published on Open.EFSA.[7]

According to Art. 32c(2) of Regulation (EC) No 178/2002 and to the Decision of EFSA's Executive Director laying down the practical arrangements on pre-submission phase and public consultations, EFSA carried out a public consultation (PC-1484) on the non-confidential version of the technical dossier from 28 May to 18 June 2025 for which no comments were received.

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) ADME; (vi) toxicological information; and (vii) allergenicity assessment.

2.2 | Methodologies

The assessment follows the methodology set out in the EFSA guidance on NF applications (EFSA NDA Panel, 2021a) 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) 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 Delegated Regulation (EU) 2016/127[11] (as regards the specific compositional and information requirements for IF and FOF and as regards requirements on information relating to infant and young child feeding).

This assessment concerns only the risks that might be associated with consumption of the NF under the proposed conditions of use and is not an assessment of the efficacy of the NF with regard to any claimed benefit. This assessment also is not an assessment of whether the NF is suitable as stipulated by Regulation (EU) No 609/2013.[8]

[4]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.

[5]Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–48.

[6]Decision available at: https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements.

[7]The non-confidential version of the dossier has been published on Open.EFSA and is available at the following link.

[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.

[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.

3 | ASSESSMENT

3.1 | Introduction

The NF, which is the subject of the application, contains 3′-SL sodium salt as the primary constituent (≥ 88% w/w dry matter (DM)). The Panel notes that although the 3′-SL sodium salt is the major component of the NF, it also contains D-lactose, sialic acid, 3′-sialyllactulose and a small fraction of other related saccharides. 3′-SL has been identified as a relevant component of the complex fraction of oligosaccharides naturally occurring in human milk, also denominated as human milk oligosaccharides (HMOs). 3′-SL is a sialylated (acidic) trisaccharide composed of D-glucose, D-galactose and NANA (hereinafter also referred to as 'sialic acid').

3′-SL (sodium salt), when produced by fermentation with genetically modified strains of *E. coli* K-12 DH1, *E. coli* BL21 (DE3) or *E. coli* W (ATCC 9637), is already authorised and included in the EU list of NFs. The NF is produced by fermentation with *E. coli* K-12 MG1655 INB-3SL_01, a genetically modified strain of *E. coli* K K-12 MG1655 (ATCC 700926).

The applicant applies for the same uses and use levels as those already authorised for 3′-SL sodium salt produced with genetically modified strains of *E. coli* K-12 DH1, *E. coli* BL21 (DE3) and *E. coli* W (ATCC 9637) included in the Union list of NFs (Commission Implementing Regulation 2017/2470). 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 3′-SL sodium salt (≥ 88% w/w DM), but it also contains D-lactose (≤ 5.0% w/w DM), sialic acid (≤ 5.0% w/w DM), 3-sialyllactulose (≤ 5.0% w/w DM) and a small fraction of other related saccharides (sum of other carbohydrates ≤ 3.0% w/w DM). It is produced by fermentation with a genetically modified strain (*E. coli* K-12 MG1655 INB-3SL_01) of *E. coli* K-12 MG1655 (ATCC 700926). The main component is the sodium salt of 3′-SL (Neu5Ac-α-(2–3)-Gal-β-(1–4)-Glc), in which sodium N-acetyl-D-neuraminate is linked through an α-(2–3) bond to D-galactose (Gal), which is linked through a β-(1–4) bond to D-glucose (Glc), in its α- and β-anomeric forms (Table 1 and Figure 1). 3′-SL is a regioisomer of 6′-SL, which contains the same monosaccharide moieties as those present in 3′-SL but with the linkage between N-acetyl-D-neuraminic acid (Neu5Ac) and D-galactose being α-(2–6) instead of α-(2–3).

TABLE 1 Chemical identity of 3′-SL sodium salt.

Chemical substance	
Chemical (IUPAC) name	Sodium; (2*S*,4*S*,5*R*,6*R*)-5-acetamido-2-[(2*R*,3*S*,4*S*,5*R*,6*S*)-3,5-dihydroxy-2-(hydroxymethyl)-6-[(2*R*,3R,4*R*,5*R*)-4,5,6-trihydroxy-2-(hydroxymethyl)oxan-3-yl]oxyoxan-4-yl]oxy-4-hydroxy-6-[(1*R*,2*R*)-1,2,3-trihydroxypropyl] oxane-2-carboxylate
Common name	3′-Sialyllactose, sodium salt
Abbreviations	3′-SL, sodium salt
Alternative chemical names	• N-Acetyl-α-D-neuraminyl-(2 → 3)-β-D-galactopyranosyl-(1 → 4)-D-glucopyranose, sodium salt • 3′-SL sodium salt • 3′-N-acetylneuraminyl-D-lactose sodium salt • α-D-Neu5Ac-(2 → 3)-β-D-Gal-(1 → 4)-D-Glc sodium salt
CAS Number	128596-80-5 (sodium salt)/35890-38-1 (acid)
Molecular formula	$C_{23}H_{38}NO_{19}Na$
Molecular mass	655.53 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 structure of 3′-SL, the major constituent of the NF.

The structure of 3′-SL was determined by mono-dimensional (1D) nuclear magnetic resonance (NMR) spectroscopy, including ^{1}H and ^{13}C spectra, and two-dimensional (2D) ^{13}C-^{1}H HSQC (heteronuclear single quantum coherence) NMR spectroscopy, by comparison to a commercially available authentic specimen.[12] The relevant coupling constants measured by ^{1}H NMR together with the correlations evidenced on the 2D NMR spectra confirmed: (i) the α-(2″-3′) glycosidic bond between Neu5Ac (C-2″) and the D-galactose (Gal-C-3′) moiety of D-lactose; (ii) the β-(1′-4) link between the D-galactose

[12] 3′-SL isolated from human milk.

(Gal-C-1′) and D-glucose (Glc-C-4) moieties of D-lactose; and (iii) the β configuration of the Gal unit. Correlations in the NMR spectra were identified and compared to those previously reported for 3′-SL (Kjærulff, 2014; Platzer et al., 1989).

The molecular structure of 3′-SL was corroborated by ultra performance liquid chromatography–tandem mass spectrometry (UPLC–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 3′-SL α-(2″-3′) and 6′-SL α-(2″-6′).

The identity of 3′-SL was also corroborated by ultra-high performance liquid chromatography coupled with a refraction index detector (UPLC–RI) by comparison to a commercially available high-purity analytical standard.

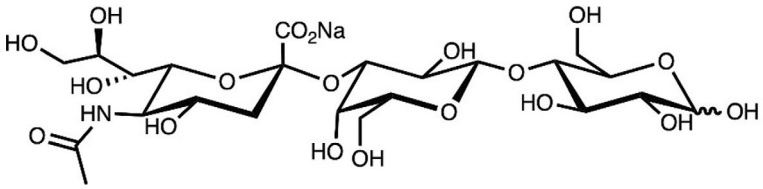


FIGURE 1 Chemical structure of 3′-SL sodium salt (EFSA NDA Panel, 2022a).

On the basis of the spectroscopic and chromatographic evidence, the Panel considers that the 3′-SL present in the NF produced by fermentation with a genetically modified strain (*E. coli* K-12 MG1655 INB-3SL_01) of *E. coli* K-12 MG1655 (ATCC 700926) is identical to the 3′-SL 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 (contract manufacturer) that is FSSC (Food Safety System Certification) 22,000 and ISO 9001:2015 certified.

The NF is produced by fermentation with a genetically modified strain (*E. coli* K-12 MG1655 INB-3SL_01) of *E. coli* K-12 MG1655 (ATCC 700926) using raw materials and processing aids suitable for food use. The production microorganism is cultured under sterile conditions in a chemically defined nutrient medium and uses sucrose and lactose to synthesise 3′-SL, which is partly excreted into the medium. Additional intracellular 3′-SL is released into the medium after pasteurisation. The production microorganism is removed from the fermentation medium by filtration at the end of the fermentation process. A series of downstream separation, purification and concentration steps are used to obtain a powdered mixture mainly composed of 3′-SL sodium salt. The NF has been produced at pilot scale, but data provided by the applicant allow the Panel to consider that upscaling the production process will not affect the composition of the NF.

The genetically modified production strain *E. coli* K-12 MG1655 INB-3SL_01 is a derivative of the parental strain *E. coli* K-12 MG1655 (ATCC 700926), with the genotype F-lambda- *ilvG- rfb-50 rph-1*. The complete genome of *E. coli* K-12 MG1655 INB-3SL_01 was sequenced and compared to the reference genome of *E. coli* K-12 MG1655. The whole genomes of *E. coli* K-12 and other closely derivative strains, including *E. coli* K-12 MG1655, 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; USEPA, 1997; ZKBS, 2021).

The production strain has been deposited at the Belgian Coordinated Collection of Microorganisms (BCCM), GeneCorner Plasmid Collection (BCCM/LMBP 12731). A detailed description of the genetic modification steps applied to the parental strain *E. coli* K-12 MG1655 (ATCC 700926) to obtain the production strain *E. coli* K-12 MG1655 INB-3SL_01 has been provided by the applicant. The absence of 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 five 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 3′-SL sodium salt as main component (92.61% w/w DM[13]). The remaining constituents[14] include D-lactose (0.89% w/w DM), sialic acid (1.26% w/w DM), 3′-sialyllactulose (0.07% w/w DM) and a small fraction of other related saccharides (sum of other quantified carbohydrates: 0.88% w/w DM).

With regards to physico-chemical properties, the NF can be described as a white powder. The solubility in water of one batch of the NF was measured in triplicate, 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 432.81 g/L. Therefore, the NF is considered highly water-soluble.

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

[13]Average content in five batches of the NF.

[14]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 produced with *E. coli* K-12 MG1655 INB-3SL_01.

	Batches of the NF					
Parameters	**A**	**B**	**C**	**D**	**E**	**Analytical method (technique)**
Composition						
3′-SL sodium salt (% DM)	93.67	91.64	90.98	94.26	92.49	UPLC–RI (validated internal method)
D-Lactose (% DM)	0.54	1.05	1.05	0.78	1.05	UPLC–RI (validated internal method)
Sialic acid (% DM)	1.12	0.89	2.42	0.64	1.24	UPLC–RI (validated internal method)
Sum of 3′-SL sodium salt, sialic acid and D-lactose (% DM)	95.33	93.57	94.44	95.68	94.78	Calculation
3′-Sialyllactulose (% DM)	0.29	< 0.01	< 0.01	< 0.01	< 0.01	UPLC–RI (validated internal method)
Sum of other carbohydrates (% w/w DM)[a]	0.20	2.19	1.21	0.53	0.29	UPLC–RI (validated internal method)
Water (% w/w)	6.7	6.3	5.0	5.4	4.5	Karl Fischer titration (volumetric titration)
Protein (% w/w)	0.0027	< 0.0025	< 0.0025	< 0.0025	< 0.0025	Roti Nanoquant (Bradford assay, spectrophotometry)
Total ash (% w/w)	7.62	7.36	7.44	6.95	8.04	NEN 6810 (500–550°C) (gravimetry)
pH (10% solution, 20°C)	5.28	4.74	4.63	4.73	5.64	Potentiometry (internal method)
Sodium (% w/w)	3.33	3.27	3.28	3.38	3.44	ICP–OES (validated internal method)
Chloride (% w/w)	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	Method (colorimetric) derived from ISO 2918 (Discrete Analyzer)
Metals and other elements						
Arsenic (mg/kg)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	ICP–MS (validated internal method)
Cadmium (mg/kg)	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	ICP–MS (validated internal method)
Lead (mg/kg)	< 0.01	< 0.01	< 0.01	< 0.01	0.016	ICP–MS (validated internal method)
Mercury (mg/kg)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	FIMS (validated internal method)
Contaminants						
Aflatoxin M1 (μg/kg)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	LC–MS/MS (validated internal method)
Aflatoxin B1 (μg/kg)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	LC–MS/MS (validated internal method)
Aflatoxin B2 (μg/kg)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	LC–MS/MS (validated internal method)
Aflatoxin G1 (μg/kg)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	LC–MS/MS (validated internal method)
Aflatoxin G2 (μg/kg)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	LC–MS/MS (validated internal method)
Aflatoxins B1 + B2 + G1 + G2 (μg/kg)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	LC–MS/MS (validated internal method)
Ochratoxin A (μg/kg)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	LC–MS/MS (validated internal method)
Microbial parameters						
Total aerobic plate count at 30°C (CFU/g)	< 100	< 100	< 100	< 100	< 100	ISO 4833 (colony count)
Yeast and moulds (CFU/g)	< 10	< 10	< 10	< 10	< 10	ISO 21527-2 (colony count)
Coliforms at 30°C (CFU/g)	< 10	< 10	< 10	< 10	< 10	ISO 4832 (colony count)

TABLE 2 (Continued)

Parameters	Batches of the NF					Analytical method (technique)
	A	B	C	D	E	
Coliforms at 30°C (in 10 g)[b]	–	–	–	–	ND	ISO 4832 (detection or qualitative method)
Enterobacteriaceae 37°C (in 10 g)	ND	ND	ND	ND	ND	ISO 21528-1 (detection or qualitative method)
Enterobacteriaceae 37°C (CFU/g)	< 10	< 10	< 10	< 10	< 10	BRD 07/24-11/13 (colony count)
Salmonella spp. (in 25 g)	ND	ND	ND	ND	ND	ISO 6579 (detection or qualitative method)
Cronobacter spp. (in 25 g)	ND	ND	ND	ND	ND	ISO 22964 (detection or qualitative method)
Listeria monocytogenes (in 25 g)	ND	ND	ND	ND	ND	AFNOR BRD 07/16-01/09 (detection or qualitative method)
Bacillus cereus (CFU/g)	< 10	< 10	< 10	< 10	< 10	ISO 7932 (colony count)
Endotoxins (EU/g)	2.40	1.65	7.70	27	19.5	Ph. Eur. 2.6.14 (LAL kinetic chromogenic assay)

Abbreviations: –, Not analysed; 3′-SL, 3′-Sialyllactose; AFNOR BRD, Association Française de Normalisation, Base de Référence Documentaire (French Standardization Association, Documentary Reference Base); CFU, colony forming unit; DM, dry matter; EU, endotoxin unit; FIMS, flow injection mercury system; ICP–MS, inductively coupled plasma–mass spectrometry; ICP–OES, inductively coupled plasma-optical emission spectrometry; ISO, International Organization for Standardization; LAL, limulus amebocyte lysate; LC–MS/MS, liquid chromatography–tandem mass spectrometry; ND, not detected; NEN, Nederlandse Norm (Dutch Standard); Ph. Eur., European Pharmacopoeia; UPLC–RI, ultra-high performance liquid chromatography–refractive index; w/w, weight per weight.

[a]Sum of other carbohydrates (detected by UPLC-RI and identified by Direct Infusion-Mass Spectrometry) includes sucrose, trisaccharide/GOS DP3 (galacto-oligosaccharide with a degree of polymerisation of 3), 3′-sialylated disaccharide/GOS DP2 (galacto-oligosaccharide with a degree of polymerisation of 2) and 3′-sialylated lactobionic acid.

[b]This parameter has been included in the Table for completeness.

3.4.1 | Stability

Stability of the NF

The stability studies were conducted with the NF produced with *E. coli* K-12 MG1655 INB-3SL_01. The applicant provided interim results (up to 36 months) for a 5-year (real-time) stability study at 25°C and 60% relative humidity (RH) on five batches of the NF. In addition, a 6-month accelerated stability study at 40°C and 75% RH was also conducted on five batches of the NF. The batches were analysed for 3′-SL, water content and main microbiological parameters (total aerobic plate count, yeasts and moulds, Enterobacteriaceae, *Cronobacter* spp., *Salmonella* spp., *Listeria monocytogenes* and *Bacillus cereus*). The pH was also measured in samples stored for 36 months at 25°C and 60% RH.

No significant changes in the monitored parameters were observed over the storage period under normal and accelerated conditions, and they remained within specifications. The applicant proposed a 5-year shelf-life under real-time conditions for the NF.

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

Stability in food matrices

The applicant tested the stability of the NF produced with *E. coli* K-12 MG1655 INB-3SL_01 in pasteurised milk (14 days at 4°C), pasteurised apple juice (1 month at 4°C) and IF (24 months at 20°C). These food categories represent some of the intended uses and reflect different processing and storage conditions relevant for those intended uses. The applicant demonstrated that 3′-SL is stable in these foods over periods representing the typical shelf-life for the respective products.

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: 3′-SL (sodium salt) is a white to off-white powder produced by microbial fermentation and subsequently retrieved, purified and concentrated	
Source: The production strain is a genetically modified strain (*Escherichia coli* K-12 MG1655 INB-3SL_01) of the parental strain *E. coli* K-12 MG1655 (ATCC 700926)	
Parameter	**Specification**
Composition	
3′-Sialyllactose sodium salt (% w/w DM)	≥ 88
D-Lactose (% w/w DM)	≤ 5.0
Sialic Acid (% w/w DM)	≤ 5.0
Sum of 3′-SL sodium salt, sialic acid and lactose (% w/w DM)	≥ 90
3′-Sialyllactulose (% w/w DM)	≤ 5.0
Sum of other carbohydrates[a] (% w/w DM)	≤ 3.0
Water (% w/w)	≤ 8.0
Protein (% w/w)	≤ 0.01
Sodium (% w/w)	2.5–4.5
Chloride (% w/w)	≤ 1.0
Total ash (% w/w)	≤ 9.0
pH (10% solution, 20°C)	4.0–6.5
Metals and other elements	
Arsenic	≤ 0.2 mg/kg
Cadmium	≤ 0.1 mg/kg
Lead	≤ 0.05 mg/kg
Mercury	≤ 0.1 mg/kg
Contaminants	
Aflatoxin M1	≤ 0.025 μg/kg

TABLE 3 (Continued)

Microbial parameters	
Aerobic plate count (CFU/g)	≤ 1000
Yeasts and moulds (CFU/g)	≤ 100
Enterobacteriaceae (in 10 g)	ND
Salmonella spp. (in 25 g)	ND
Cronobacter spp. (in 10 g)	ND
Listeria monocytogenes (in 25 g)	ND
Bacillus cereus (CFU/g)	≤ 50
Endotoxins (EU/mg)	≤ 10

Abbreviations: 3′-SL: 3′-Sialyllactose, CFU: colony forming units, DM: dry matter, EU: endotoxin units, ND: not detected, w/w: weight per weight.

[a]Sum of other carbohydrates (detected by UPLC–RI and identified by direct infusion–mass spectrometry) includes sucrose, trisaccharide/GOS DP3 (galacto-oligosaccharide with a degree of polimerisation of 3), 3′-sialylated disaccharide/GOS DP2 (galacto-oligosaccharide with a degree of polimerisation of 2) and 3′-sialylated lactobionic acid.

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.

3′-SL, the major constituent of the NF, is a sialylated oligosaccharide found in human milk (Soyyılmaz et al., 2021; Thurl et al., 2017). The NF is produced by fermentation with *E. coli* K-12 MG1655 INB-3SL_01, a genetically modified strain of *E. coli* K-12 MG1655 (ATCC 700926). 3′-SL sodium salt is already authorised as a NF when produced with other genetically modified strains of *E. coli* (see Section 1.2).

3.7 | Proposed uses and use levels and anticipated intake

3.7.1 | Target population

The target population proposed by the applicant is the general population, except for FS, for which the target population is individuals above 3 years of age.

3.7.2 | Proposed uses and use levels

The applicant does not intend to amend the uses and use levels already authorised for 3′-SL sodium salt when produced by 'microbial source' (EFSA NDA Panel, 2020a; Implementing Regulation (EU) 2021/96[2] and (EU) 2017/2470[3]). The Panel notes that other uses and use levels for 3′-SL sodium salt when produced according to different production processes are also authorised and included in the Union list of NFs. Therefore, the NF would be consumed to the same extent as the already authorised 3′-SL sodium salt and no estimate of the intake has been carried out.

3.7.3 | Precautions and restrictions of use

The same restrictions of use as those already authorised for other production processes apply, i.e. excluding the use as FS in infants and young children and FS are not intended to be used if other foods with added 3′-SL sodium salt are consumed on the same day.

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

As mentioned by the applicant and reported in previous EFSA opinions (e.g. EFSA NDA Panel, 2015a, 2019a, 2022a) HMOs, including 3′-SL, 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 mostly fermented in the colon by intestinal microbiota while a fraction is excreted

unchanged in the faeces and a small fraction is found in the urine (EFSA NDA Panel, 2022a). In addition, there are no indications that the absorption of 3′-SL or other structurally related mono-and oligosaccharides (e.g. sialic acid) from the NF, differs from that of similar components in human milk.

Nevertheless, the applicant conducted toxicokinetic analysis with the NF as part of the 90-day toxicity study. In this study four satellite groups of rats (3 males and 3 females per group) received the NF daily via oral gavage (dose levels of 0, 1500, 3000 or 5000 mg/kg bw per day – Study Report, 2022; see also Section 3.10.3). Blood samples for the determination of plasma levels of 3′-SL were collected at the end of the treatment period (1, 2, 4, 6 and 24 h after administration). Urine samples were also collected from rats kept for at least 24 h in metabolic cages. Systemic exposure to 3′-SL up to 4 or 6 h after administration was achieved in all treated animals. The C_{max} of 3′-SL in plasma was generally observed 1 h after the administration while some animals showed a second peak of plasma concentration observed 6 h after administration. The AUC_{0-t} ranged from about 4000–41,000 ng*h/mL. In the range of administered doses, the C_{max} and AUC_{0-t} increased proportionally with increasing doses at 1500 and 3000 mg/kg and more than dose proportionally between 3000 and 5000 mg/kg per day (likely due to experimental variability according to the author). Higher systemic exposure to 3′-SL and consequently occurrence in urinary levels was generally observed in male rats compared to female rats. However, the amount of 3′-SL recovered in urine collected over a period of 24 h after administration both from males and females was below 0.5% of the daily exposure (calculated as percentage of the administered doses). It is noted that 3′-SL was also found in blood and urine samples from control rats and were considered from endogenous origin as previously reported (EFSA NDA Panel, 2026; Vazquez et al., 2017).

In conclusion, 3′-SL when administered in the form of 3′-SL sodium salt was systemically available in all animals.

3.9 | Nutritional information

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

The NF contains other carbohydrates individually present at low concentrations (from 0.01% to about 2%, see Table 2). D-Lactose (about 1%) is the most abundant molecule in human milk (~7%) and its monomers, D-glucose and D-galactose, are normal constituents of human milk. Sialic acid (< 2.5%) is a building block of HMO (sialylated oligosaccharides) and a normal component found in human milk (Goehring et al., 2014). Only traces of other related oligosaccharides (e.g. 3′-sialyllactulose) can be detected in the NF.

The Panel notes that the NF, being a sodium salt, may contribute to the daily sodium intake. Considering that the NF would be consumed to the same extent as the already authorised 3′-SL sodium salt and having a similar sodium content, the same considerations apply (EFSA NDA Panel, 2020a).

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) principles of Good Laboratory Practice (GLP) (OECD, 1998) and in accordance with OECD test guidelines (TG) No 471, 487 and 408. The Panel notes that the studies were all conducted with the same batch of the NF produced with *E. coli* K-12 MG1655 INB-3SL_01. An additional non-GLP regulated in vivo repeated dose-range finding (DRF) study was also carried out. The studies which were claimed proprietary by the applicant are listed in Table 4.

TABLE 4 List of toxicological studies with the NF provided by the applicant.

Reference	Type of study	Test system	Dose (3′-SL sodium salt)
Study No. 20/066-007M Study report (2020)	Bacterial reverse mutation test (GLP, OECD TG 471) (OECD, 1997)	*Salmonella* Typhimurium TA98, TA100, TA1535 and TA1537. *Escherichia coli* WP2 uvrA	Up to 5000 µg/plate (absence and presence of S9-mix)
Study No. 20258017 Study report (2021a)	*In vitro* mammalian cell micronucleus test (GLP, OECD TG 487) (OECD, 2016)	Human lymphocytes	Up to 2000 µg/mL (absence and presence of S9-mix)
Study No. 48322 RSR Study report (2021b)	Preliminary toxicity study by oral route (21-day)	Juvenile Sprague–Dawley Crl:CD(SD) rats	0, 3000, 4000 or 5000 mg/kg bw per day (oral gavage from PND 7)
Study No. 48323 RSR Study report (2022)	90-day toxicity study by oral route (GLP, OECD TG 408 (2018))	Juvenile Sprague–Dawley Crl:CD(SD) rats	0, 1500, 3000 or 5000 (and 5000 FOS) mg/kg bw per day (oral gavage from PND 7).

Abbreviations: bw, body weight; FOS, fructo-oligosaccharides; GLP, Good Laboratory Practice; OECD, Organisation for Economic Co-operation and Development; PND, post-natal day; S9-mix, liver microsomal fraction; SD, Sprague–Dawley; TG, test guideline.

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. A chromosomal aberration assay in human lymphocytes was also carried out (Table 4).

The *in vitro* assessment of the mutagenic potential of the NF (Study Report, 2020) was performed with *S*. Typhimurium, strains TA98, TA100, TA1535 and TA1537, and *E. coli* WP2 uvrA. A preliminary mutagenicity test was conducted with the plate incorporation method at six different concentrations from 10 to 5000 µg NF/plate, followed by a confirmatory test applying the pre-incubation and plate incorporation methods at concentrations ranging from 15.81 to 5000 µg NF/plate, either in the presence or absence of metabolic activation (S9-mix). No precipitate and no cytotoxicity were observed following exposure to any concentrations of the NF in any strain. 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 the NF at any concentration in either experiment with or without S9 mix.

In the *in vitro* mammalian cell micronucleus test in human lymphocytes (Study Report, 2021a), concentrations of 500, 1000 and 2000 µg NF/mL were tested in the main study in the presence (3 h treatment) and absence (3 and 24 h treatments) of metabolic activation (S9-mix). The main assay was preceded by a dose-range finding test (concentrations ranging from 63 to 2000 µg NF/mL). No cytotoxicity or precipitation was observed, and the NF did not induce a statistically significant or biologically relevant increase in the number of binucleated cells with micronuclei in cultured human lymphocytes in any of the test substance concentrations.

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

3.10.2 | Subacute toxicity

The applicant provided a 21-day repeated dose pilot toxicity study where three groups of each eight Crl:CD (SD) neonatal (from PND 7) male and eight neonatal female rats were given 3000, 4000 or 5000 mg NF/kg bw per day by gavage. Another group of eight males and eight females received the vehicle, sterile water for injection, under the same experimental conditions and acted as a control group (Study Report, 2021b). Clinical observations were registered and haematological and blood chemistry investigations at the end of the treatment period were performed (limited to two to four rats/sex per dose group). Gross examination of principal organs at necropsy (PND 28) was performed; selected organs were weighed. Mortality (1 male and 1 female in the high-dose group) in PND 10 and 19 was recorded; a cause of death could not be determined. One male and one female in the low-dose group died in the last week of treatment and mortality was related to technical issue during the dosing procedure. The treatment was well tolerated and no clinical signs attributable to the treatment were noted. A minimal effect (decrease) in body weight in high-dose males coupled with a slight decrease in food consumption was recorded throughout the dosing period. At laboratory examination, despite a few statistically significant differences noted in low- and mid-dose groups (e.g. slight decrease in red blood cells and increase in reticulocytes (%)), there were no findings judged to be of biological relevance. According to the author no organ weight changes considered to be treatment-related were noted. However, statistically significant variations in a few organs (e.g. brain, liver, testis) in the males receiving the high dose were recorded. No findings at post-mortem examination were noted. The high dose of 5000 mg NF/kg bw per day was selected as the high dose to be used in the subsequent 90-day study.

3.10.3 | Subchronic toxicity

In the 90-day study, four groups of each 10 Crl:CD (SD) neonatal male and 10 neonatal female rats were administered by gavage a dose of 0 (vehicle, sterile water for injection), 1500, 3000 and 5000 mg NF/kg bw per day once daily for 90 consecutive days, starting from PND 7. An additional reference control group received FOS (fructo-oligosaccharides) at 5000 mg/kg bw per day under the same conditions. Additional five rats per sex in the control and high-dose NF groups were also dosed once daily for 90 days and then observed over a 5-week recovery period to assess the reversibility of any changes observed in the dosing phase (Study Report, 2022). Rats from the satellite groups (three males and three females per group) for the determination of urine and plasma levels of the NF were also included in the study (see also Section 3.8). This study has been designed based upon the OECD TG408 (OECD, 1998), with some adaptations (i.e. use of juvenile animals) to consider the requirements for toxicity testing of new chemical entities for use in the paediatric population (as was suggested by US FDA, 2006 and EMEA, 2008). In addition to the standard examinations (including functional observational battery (FOB)) and in consideration of the age of the rats, specific observations were carried out, including pre-weaning development, tibia length measurement, sexual maturation and oestrous cycle monitoring. Seminology investigations (e.g. spermatozoa count) were also performed at the end of the treatment period. Blood and urine samples for haematology, coagulation, blood biochemistry (including determination of thyroid hormone levels) and urinary investigations were collected from all animals at the end of the treatment period. A complete post-mortem examination was performed in all rats while microscopic examination was carried out in control, reference and high-dose groups.

Mortality was observed in the mid-dose group: one male and one female, were euthanised on human ground on PND 25 and 28, respectively (poor general conditions were due to technical issue during the gavage procedure (M) or caecal

necrosis (F)). One female rat from the high-dose group was found dead after blood sampling procedure on PND 94. In addition, one male and one female from reference group were found dead on PND 19 and 31, no clear cause of death was determined. While no relevant clinical signs in the treated groups were noted, in the reference group yellowish faeces and reddish and soiled urogenital areas were observed in the first 3 weeks of the treatment period in both sexes. No changes in body weight and food consumption throughout the study considered to be treatment-related were recorded. However, statistically significant body weight increase in the mid-dose male group, in the high-dose male group (limited to the recovery period) and in the reference group (both sexes, with increased food consumption) starting from the second month of treatment was noted. Although sporadic statistically significant variations were noted (females), there were no impairment of learning or memory function considered related with the test or the reference item. The same applies for motor activity and for effects on long bone growth. No biologically relevant differences in the age or body weight at which the males and females attained physical signs of sexual maturation, were observed. Oestrous cycles were unaffected by the NF administration and no relevant changes were noted on sperm production, morphology, count and motility. No ophthalmological findings were recorded. Statistically significant differences in some haematological, biochemical, hormonal (thyroid) and urinary parameters (mostly in males, see details in Appendix A) were noted. According to the author, the statistically significant differences between control and animals treated with 3′-SL were not attributed to the treatment as they were of low magnitude, not dose-related, observed in one sex only, sometimes also noted in the reference group and they are overall considered as not toxicologically relevant.

At post-mortem examination, no macroscopic findings were observed that were considered to be related to the administration of 3′-SL. Very few differences between test item-treated groups and controls in organ weight were noted and interpreted as incidental. At histological examination a few findings were noted and were also considered as incidental, as they also occurred in controls, were of low incidence, had no dose–response relationship, or are common background findings for the species under the laboratory conditions applied.

The Panel considers that no adverse effects were observed in this study up to the highest tested dose of 5000 mg NF/kg bw per day.

3.10.4 | Human data

No human intervention studies conducted with the NF have been provided by the applicant.

However, the applicant referred to a paper describing a randomised, controlled, multicentre, double-blinded, parallel feeding growth and tolerance study conducted in infants receiving an IF containing a mixture of five HiMOs (EFSA NDA Panel, 2025a, 2026; Lasekan et al., 2022). 3′-SL accounted for 0.2 g/L in the IF. Other added HiMOs were LNT, 3-FL, 6′-SL and 2′-FL, the latter being the most represented HiMO (3.0 g/L). All five HiMOs were added according to their average concentrations in human milk. Three groups of healthy term infants (at ≤ 14 days of age) were enrolled. One group was fed with the experimental formula containing the five HiMOs, the second group with a control milk-based IF lacking HiMO and the third group was exclusively breastfed until approximately 4 months of age. The study demonstrated that the IF containing the HiMO mixture did not lead to impaired growth, was gastrointestinally tolerated and safe to be used by healthy term infants.

The Panel considers the information provided by the applicant as supportive for the safety assessment of 3′-SL sodium salt.

3.11 | Allergenicity

The protein content of the NF is ≤ 0.01% w/w as indicated in the specifications (Table 4). The applicant did not identify an allergenic potential of proteins introduced because of the genetic modification of the *E. coli* K-12 MG1655 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 bioinformatic study was conducted on the Allergen Online database. The criterion used for identifying sequence homology with known allergens was that of considering 'higher than 35% identity in a sliding window of 80 amino acids'.

The Panel considers that the likelihood of allergenic reactions to the NF is low.

4 | DISCUSSION

The NF is a powdered mixture mainly composed of 3′-SL sodium salt, but it also contains D-lactose, sialic acid, 3′-sialyllactulose and a small fraction of other related saccharides. 3′-SL sodium salt, when produced by fermentation with genetically modified strains of *E. coli* K-12 DH1, *E. coli* BL21 (DE3) or *E. coli* W (ATCC 9637) is already authorised and included in the EU list of NFs. The NF is produced by fermentation with *E. coli* K-12 MG1655 INB-3SL_01, which is a genetically modified strain of *E. coli* K-12 MG1655 (ATCC 700926).

Of note is that the application pertains to a change in the production process and specifications, while the target population, conditions of use and consequently the anticipated intake of 3′-SL sodium salt, remain unchanged. In addition, the

same restrictions of use as those already authorised apply, i.e. excluding the use as FS in infants and young children and FS are not intended to be used if other foods with added 3′-SL sodium salt are consumed on the same day.

The submitted toxicity studies did not raise safety concerns. No findings considered to be toxicologically relevant were observed in a subchronic toxicity study performed in juvenile SD rats up to the highest dose tested of 5000 mg NF/kg bw per day. It is also 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 their intake by breastfed infants and the estimated intake of NF components under the proposed conditions of use. Given that the NF would be consumed to the same extent as the already authorised 3′-SL sodium salt and taking into account the intrinsic nature of HiMOs with their limited absorption, the absence of toxicologically relevant effects and considering that breastfed infants are naturally exposed to this substance, the Panel considers that the consumption of 3′-SL in the NF produced by fermentation with a genetically modified strain (*E. coli* K-12 MG1655 INB-3SL_01) of *E. coli* K-12 MG1655 (ATCC 700926) under the proposed conditions of use does not raise safety concerns.

5 | CONCLUSIONS

The Panel concludes that the NF, which is composed of 3′-SL (sodium salt) 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 as listed in Appendix B.

ABBREVIATIONS

1D	mono-dimensional
2′-FL	2′-fucosyllactose
2D	two-dimensional
3′-SL	3′-sialyllactose
3-FL	3-fucosyllactose
6′-SL	6′-sialyllactose
ADME	absorption, distribution, metabolism and excretion
AFNOR BRD	Association Française de Normalisation, Base de Référence Documentaire (French Standardization Association, Documentary Reference Base)
ATCC	American Type Culture Collection
AUC	area under the curve
BCCM	Belgian Coordinated Collection of Microorganisms
BIOHAZ Panel	EFSA Panel on biological hazards
bw	body weight
CAS	Chemical Abstracts Service
CFU	colony forming units
Cmax	maximum concentration
Crl:CD (SD)	Sprague–Dawley rats
DFL	difucosyllactose
DM	dry matter
DNA	deoxyribonucleic acid
DP	degree of polimerisation
DRF	dose-range finding
EPA	US Environmental Protection Agency
F	female
FDA	US Food and Drug Administration
FEEDAP Panel	EFSA Panel on Additives and Products or Substances used in Animal Feed
FIMS	flow injection mercury system
FOB	functional observational battery
FOF	follow-on formula
FOS	fructo-oligosaccharides
FS	food supplements
FSMP	food for special medical purposes
FSSC	Food Safety System Certification
Gal	D-galactose

Glc	D-glucose
GlcNAc	N-acetyl-D-glucosamine
GLP	Good Laboratory Practices
GMO	genetically modified organisms
GMP	Good Manufacturing Practice
GOS	galacto-oligosaccharides
HACCP	Hazard Analysis Critical Control Points
HiMO	human identical milk oligosaccharides
HMO	human milk oligosaccharides
HSQC	heteronuclear single quantum coherence
ICP–MS	Inductively coupled plasma–mass spectrometry
ICP–OES	inductively coupled plasma–optical emission spectrometry
IF	infant formula
INB	Inbiose
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
LAL	Limulus amebocyte lysate
LC–MS/MS	liquid chromatography–tandem mass spectrometry
LNFP	lacto-N-fucopentaose
LNnT	lacto-N-neotetraose
LNT	lacto-N-tetraose
M	male
NANA, Neu5Ac	N-acetyl-D-neuraminic acid
ND	not detected
NDA Panel	EFSA Panel on Nutrition, Novel Foods and Food Allergens
NEN	Nederlandse Norm (Dutch Standard)
NF	Novel food
NMR	nuclear magnetic resonance
OECD	Organisation for Economic Co-operation and Development
PC	public consultation
Ph.Eur.	European Pharmacopoeia
PND	post-natal day
QPS	qualified presumption of safety
Rf	retention factor
RH	relative humidity
RI	reference item
S9-mix	S9 metabolic activation mixture
TG	Test guideline
UPLC–MS/MS	ultra-high performance liquid chromatography–tandem mass spectrometry
UPLC–RI	ultra-high performance liquid chromatography–refraction index detection
US	United states
w/w	weight per weight

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APPENDIX A

90-day juvenile rat toxicity study: Laboratory examination at the end of the treatment and recovery phase (Study Report, 2022) - parameters with statistical significance

Haematology

	Males (NF mg/kg bw per day)					Females (NF mg/kg bw per day)				
Dose level	**0**	**1500**	**3000**	**5000**	**5000 RI**	**0**	**1500**	**3000**	**5000**	**5000 RI**
White blood cells (g/L)	11.16	10.21	9.21	10.44	8.71*	8.47	6.77*	7.78	6.51*	6.55*
Mean cell volume (fL)	54.7	55.0	55.5	54.3	55.3	56.6	56.0	54.7**	55.1*	56.3
Mean cell haemoglobin (pg)	18.3	18.4	18.3	18.1	18.2	19.1	19.0	18.5*	18.8	19.1
Lymphocytes (g/L)	9.14	8.49	7.21*	7.84	6.76**	7.28	5.74	6.59	5.27**	5.27**
Large unstained cells (g/L)	0.08	0.07	0.07	0.05**	0.05**	0.06	0.04	0.06	0.05	0.04*

Note: Statistically significant from control group: *$p < 0.05$, **$p < 0.01$.

Abbreviations: bw, body weight; fL, femtoliter; NF, novel food; RI, reference item (FOS: fructo-oligosaccharides).

Blood chemistry and hormone levels

	Males (NF mg/kg bw per day)					Females (NF mg/kg bw per day)				
Treatment	**0**	**1500**	**3000**	**5000**	**5000 RI**	**0**	**1500**	**3000**	**5000**	**5000 RI**
End of treatment phase										
Sodium (mmol/L)	142.9	141.6 **	142.0	140.9**	141.8	142.1	141.6	142.2	140.5**	142.1
Chloride (mmol/L)	104.7	103.9	103.9	101.1**	103.4	105.8	105.3	106.2	103.8**	104.6*
Calcium (mmol/L)	2.63	2.61	2.64	2.56**	2.63	2.66	2.61	2.61	2.59	2.63
Phosphorus (mmol/L)	2.13	2.34**	2.16	2.16	2.15	1.89	1.88	1.70*	1.76	1.78
Urea (mmol/L)	4.5	5.0*	5.0*	4.8	4.7	4.8	4.9	5.2	5.6	4.9
Creatinine (µmol/L)	26.11	28.29**	28.06*	2.48	27.66**	29.32	28.47	30.57	30.83	26.70*
Total bilirubin (µmol/L)	0.84	1.03	1.18	1.20*	1.48**	1.34	1.41	1.50	1.19	1.62
Proteins (g/L)	60.8	59.1	59.5	56.3**	57.3*	63.1	61.9	62.6	62.0	59.8
Albumin/globulin ratio	1.73	1.95**	1.87*	1.94**	1.89**	1.92	2.19**	2.07	2.03	2.08
Cholesterol (mmol/L)	2.00	1.63	1.94	1.46**	1.43**	2.15	2.03	1.97	2.03	2.12
Alkaline phosphatase (U/L)	280	276	222	222*	201*	184	139	166	139*	126*
Aspartate aminotransferase (U/L)	80	77	85	96**	85	74	87	94*	94	85
Bile acids (µmol/L)	29.65	37.90	33.62	44.39**	41.08**	23.03	42.80**	25.41	33.36**	34.91**±
HDL cholesterol (mmol/L)	0.39	0.32*	0.37	0.30**	0.31**	0.52	0.51	0.48	0.51	0.51
T3 (ng/mL)	0.32	0.50**	0.47**	0.43**	0.48**	0.41	0.41	0.39	0.39	0.42
T4 (ng/mL)	41.83	49.58**	53.87**	48.95**	50.24**	28.62	33.96	32.18	35.64**	35.83*

	Males (NF mg/kg bw per day)		Females (NF mg/kg bw per day)	
Treatment	**0**	**5000**	**0**	**5000**
End of recovery phase				
WBC (G/L)	11.06	9.16	8.69	4.45**
Red Blood Cells T/L	8.68	8.25**	7.94	8.26
Mean Corpuscular Volume (fL)	56.3	57.0	59.4	56.5**
Mean concentration haemoglobin (pg)	18.0	18.5	19.4	18.5*
Mean cell haemoglobin concentration (g/dL)	31.8	32.5*	32.6	32.8
Neutrophils (g/L)	1.68	1.23**	0.98	0.80
Leucocytes (G/L)	8.75	7.41	7.31	3.40**
Large unstained cells (G/L)	0.09	0.07	0.06	0.02**
Monocytes (G/L)	0.35	0.32	0.22	0.12**
Phosphorus (mmol/L)	2.0	2.19**	1.65	1.41**

(Continues)

(Continued)

	Males (NF mg/kg bw per day)		Females (NF mg/kg bw per day)	
Treatment	**0**	**5000**	**0**	**5000**
Total bilirubin (μmol/L)	0.36	0.33	0.83	1.01*
Albumin (g/L)	39	39	42	45*
Albumin/globulin ratio	1.72	1.85*	1.78	2.02*
Alkaline phosphatase (U/L)	231	147**	150	90*
Aspartate aminotransferase (U/L)	37	31	25	41*

Note: Statistically significant from control group *$p < 0.05$, **$p < 0.01$.

Abbreviations: bw, body weight; NF, novel food; U, units.

APPENDIX B

List of elements of the dossier for which data protection was requested by the applicant

Dossier section	Elements of the application dossier for which a request for data protection was filed by the applicant	Considered as pertinent for the NDA panel to reach the conclusion on the safety of the NF
Identity	Section 2.1 (identity of the novel food; pages 1–11) Annex 2.1.02 (NMR report; pages 1–9) Annex 2.1.03 (UPLC_RI validation report INBMV1001; pages 1–32) Annex 2.1.04 (UPLC–MSMS analysis 3SL; pages 1–5)	**Yes**
Production process	Section 2.2 (production process_updated ADR2; pages 1–9) Annex 2.2 (ADR2_PP; page 1) Annex 2.2.04 (GMM_description; pages 1–43) Annex 2.2.04.02 (structure helper plasmids and sequence plasmids; pages 1–18) Annex 2.2.04.03 (recombinant genes; pages 1–6) Annex 2.2.04.04 (amino acid sequences recombinant proteins; pages 1–2) Annex 2.2.04.05 (genetic information; pages 1–2) Annex 2.2.04.06 (contigs_3SL_fasta file; pages 1–1252) Annex 2.2.04.07 (PROKKA_3SL_fasta file; pages 1–567) Annex 2.2.04.08 (Deposition certificate; pages 1–5) Annex 2.2.04.09 (structure and sequence pINB-3SL_01; pages 1–2) Annex 2.2.04.10 (additional AMR INB_3SL_01; pages 1–7) Annex 2.2.04.11 (test for absence of viable cells; pages 1–18) Annex 2.2.05 (raw materials_updated ADR2; pages 1–4) Annex 2.2.06 (differences pilot vs. commercial_updated ADR2; pages 1–2) Annex 2.2.07 (HACCP plan; page 1) Annex 2.2.08 (determination of residual processing aid in 3′-SL sodium salt powder batches; pages 1–4) Annex 2.2.09 (HACCP plan_2019; page 1) Annex 2.2.10 (effect of heat treatment_3SL; pages 1–10) Protocol for the preparation of master and working cell banks (pages 1–4)	**Yes**
Composition	Section 2.3 (compositional data_updated ADR2; pages 1–14) Annex (Annex_ADR3_CD; pages 1–2) Annex 2.3.01 (ash content; pages 3–7) Annex 2.3.02 (carbohydrates; pages 2–6) Annex 2.3.03 (endotoxins; pages 3–7) Annex 2.3.04 (Enterobacteriaceae; pages 3–17) Annex 2.3.05 (pH analysis; pages 3–7) Annex 2.3.06 (microbiology; pages 3–16) Annex 2.3.07 (sodium; pages 3–12) Annex 2.3.08 (protein content; pages 3–7) Annex 2.3.09 (water content; pages 3–12) Annex 2.3.10 (heavy metals; pages 3–17) Annex 2.3.11 (mycotoxins; pages 3–22) Annex 2.3.12 (chlorides; pages 3–12) Annex 2.3.13 (absence of rDNA; pages 2–6) Annex 2.3.14 (method description results qNMR (Inbiose); pages 1–2) Annex 2.3.15 (minor carbohydrates UPLC-RI; pages 1–16) Annex 2.3.16.01 (Carbohydrates_Calculation matrix_UPLC-RI Excel sheet: 3-SL Sodium salt) Annex 2.3.16.02 (Carbohydrates_Calculation matrix_qNMR Excel sheet: qNMR 3SL_Na %DM) Annex 2.3.17.01 (determination of HMOs by LC–MSMS; pages 1–14) Annex 2.3.17.02 (validation report_LC–MSMS; pages 1–12) Annex 2.3.18 (3SL solubility report; pages 1–7) Annex 2.3.19 (3SL_Stability study_Powder 40C 70RH; pages 3–188) Annex 2.3.20 (Summary stability study 3SL 40_75RH Excel sheet: 40_75 Excel sheet: graphs 40_75 Excel sheet: microbio) Annex 2.3.21 (3SL_Stability study_Powder 25C 60RH; pages 1–334) Annex 2.3.22 (Summary stability study 3SL 25_60RH Excel sheet: 25_60 Excel sheet: graphs 25_60 Excel sheet: microbio) Annex 2.3.23 (3SL_Stability study_Liquid food matrices; pages 3–37) Annex 2.3.24 (3SL_Stability study_Infant formula; pages 3–64) Annex 2.3.25 (manufacturing of the infant formula; pages 1–4) Annex 2.3.31 (peak identification; pages 1–7) Annex 2.3.32.01 (3SLu_Production; pages 1–6) Annex 2.3.32.02 (3SLu_Identity; pages 1–2)	**Yes**
ADME	Section 2.7 (ADME; pages 1–2)	**Yes**

(Continues)

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Dossier section	Elements of the application dossier for which a request for data protection was filed by the applicant	Considered as pertinent for the NDA panel to reach the conclusion on the safety of the NF
Toxicological information	Section 2.9 (toxicological information; page 1) Subsection 2.9.2 (mutagenicity and genotoxicity studies; pages 3–6 of the Section 2.9) Subsection 2.9.3 (subchronic oral toxicity study in the rat; pages 7–15 of Section 2.9) Annex 2.9.01 (mutagenicity study 3′-SL OECD 471; pages 1–84) Annex 2.9.02 (genotoxicity study 3′SL OECD 487; pages 1–117) Annex 2.9.03 (OECD 408 formulation analyses; pages 1–64) Annex 2.9.04 (validation plasma analysis; pages 1–94) Annex 2.9.05 (validation urinalysis; pages 1–92) Annex 2.9.06 (dose-range finding study; pages 1–255) Annex 2.9.07 (OECD 408_Subchronic study report; pages 1–1665)	**Yes**
Allergenicity	Annex 2.10.01 (allergen results; pages 1–6)	**Yes**
Appendix B.3	Summary table statistically significant observations toxicity studies_DRF study 3′-SL (pages 1–6) Summary table statistically significant observations toxicity studies_Subchronic oral tox study 3′-SL (pages 1–21)	**Yes**
Other relevant information	P1512_R03_v01 (method description for monitoring HMOs in food ingredient and food matrices) (pages 1–60) P1679_R01_v01_INB_signed (evaluation of alternative filter membrane for sample preparation in HMO LC–MS method) (pages 1–33)	**Yes**