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Safety of 6′-Sialyllactose (6′-SL) sodium salt as a novel food pursuant to Regulation (EU) 2015/2283

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

Abstract

Following a request from the European Commission, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver an opinion on 6′-Sialyllactose (6′-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) 6′-SL but also contains D-lactose, sialic acid and a small fraction of other related oligosaccharides. The NF is produced by fermentation with a genetically modified strain of *Escherichia coli* K-12 DH1. The information provided on the manufacturing process, composition and specifications of the NF does not raise safety concerns. The applicant intends to add the NF in a variety of foods, including infant and follow-on formula, foods for infants and toddlers, foods for special medical purposes and food supplements. The target population is the general population. The anticipated daily intake of 6′-SL from the NF at the maximum proposed use levels is unlikely to exceed the intake level of naturally occurring 6′-SL in breastfed infants on a body weight basis. The intake of 6′-SL in breastfed infants on a body weight basis is expected to be safe also for other population groups. The intake of other carbohydrate-type compounds structurally related to 6′-SL is also considered of no safety concern. Food supplements are not intended to be used if other foods with the added NF or breast milk are consumed on the same day. The Panel concludes that the NF is safe under the proposed conditions of use.

Keywords: 6′-Sialyllactose, 6′-SL, human milk oligosaccharide, HMO, HiMO, novel food, safety

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Correspondence: nda@efsa.europa.eu

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

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1. Introduction

1.1. Background and Terms of Reference as provided by the European Commission

On 31 January 2019, the company Glycom A/S submitted a request to the European Commission in accordance with Article 10 of Regulation (EU) 2015/2283[1] to place on the EU market 6′-Sialyllactose (6′-SL) sodium salt as a novel food.

6′-Sialyllactose (6′-SL) sodium salt is intended to be used in a number of food categories.

In accordance with Article 10(3) of Regulation (EU) 2015/2283, the European Commission asks the European Food Safety Authority to provide a scientific opinion on 6′-Sialyllactose (6′-SL) sodium salt as a novel food.

2. Data and methodologies

2.1. Data

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

During the assessment, the Panel identified additional data, which were not included in the application (Goehring et al., 2014; Gorbach, 1978, Rudloff et al., 1996, 2012; Vazquez et al., 2017; Zeng et al., 2018).

Administrative and scientific requirements for NF applications referred to in Article 10 of Regulation (EU) 2015/2283 are listed in the Commission Implementing Regulation (EU) 2017/2469[2].

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

This NF application includes a request for protection of proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283. Data claimed to be proprietary by the applicant include:

- Detailed description of the production process – raw materials and schematic overview of the processing (sections of the dossier 2.b.1.1-4).
- Annexes to the dossier, which relate to the identity, production process, production microorganism, composition and specifications of the NF (annex I 'NMR analytical report', annex II 'production strain data', annex III 'production strain certificates', annex IV 'raw materials and processing aids', annex V 'certificate of analysis and batch data', annex VI 'analytical method validation report', annex VII 'Stability reports', annex VIII 'Laboratory accreditation certificates').
- Intakes assessment report (annex X to the dossier).
- Unpublished toxicological study reports:
  - 6′-Sialyllactose sodium salt – Bacterial Reverse Mutation Test (Unpublished Study report 2018a); *In vitro* Mammalian Cell Micronucleus Test (Unpublished Study report 2018b); 14-Day Toxicity Study in the Neonatal Rat (Unpublished Study report, 2018c); 90-Day Toxicity Study in the Neonatal Rat (Unpublished Study report, 2018d)
  - 3′-Sialyllactose sodium salt – Bacterial Reverse Mutation Test (Unpublished study report, 2019a); *In vitro* Mammalian Cell Micronucleus Test (Unpublished study report, 2019b); 14-Day Toxicity Study in the Neonatal Rat (Unpublished study report, 2019c); 90-Day Toxicity Study in the Neonatal Rat (Unpublished study report, 2019d)
- Appendix B.3 to the dossier referring to the summary table of statistically significant observations in toxicity studies with 6′-Sialyllactose.

[1] Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) N0 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001 (2013/0435 (COD). OJ L 327, 11.12.2015, p. 1–22.

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

2.2. Methodologies

The assessment follows the methodology set out in the EFSA guidance on NF applications (EFSA NDA Panel, 2016) and the principles described in the relevant existing guidance documents from the EFSA Scientific Committee. The legal provisions for the assessment are laid down in Article 11 of Regulation (EU) 2015/2283 and in Article 7 of the Commission Implementing Regulation (EU) 2017/2469.

This assessment concerns only risk that might be associated with consumption of the NF under the proposed conditions of use and is not an assessment of the efficacy of 6′-Sialyllactose (6′-SL) sodium salt with regard to any claimed benefit.

3. Assessment

3.1. Introduction

The NF primary constituent is the sodium salt of 6′-Sialyllactose, henceforth named '6′-SL sodium salt'. The trisaccharide 6′-Sialyllactose (6′-SL) is composed of the monomers glucose, galactose and N-acetylneuraminic acid (NANA, hereinafter also referred to as 'sialic acid'). It is one of the most abundant sialylated (acidic) oligosaccharides in the oligosaccharide fraction of human milk (HMOs – human milk oligosaccharides). The 6′-SL in the NF is obtained by fermentation and is isolated as a purified ingredient in the sodium salt form. The NF is intended to be used in foods for infants and young children (including the use as human-identical milk oligosaccharides (HiMO) in infant formulae (IF) and follow-on formulas), foods for special medical purposes, total diet replacements for weight control, food supplements, beverages and a variety of other foods (e.g. dairy products, bakery wares). The target population is the general population.

Other HiMOs, namely LNnT, 2′-FL, DFL and LNT produced by chemical synthesis or by fermentation with derivatives of *E. coli* K-12, have been assessed previously (EFSA NDA Panel, 2015, 2019a,b).

The applicant indicated that according to Regulation (EU) 2015/2283, this 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 6′-SL sodium salt, but also containing D-lactose, sialic acid and a small fraction of other related saccharides resulting in a fully characterised mixture of carbohydrates (> 99% in representative batches, see Table 1). It is produced by fermentation with a genetically modified strain of *Escherichia coli* K12 DH1 MDO. The main component is the sodium salt of Neu5Ac-(α2-6)-Gal-(β1-4)-Glc (6′-SL) in which sodium N-acetylneuraminate is linked through an α-(2-6) bond to D-galactose and linked through a β-(1-4) bond to the reducing end D-glucose, which is in equilibrium between the α- and β-anomeric forms. 6′-SL is a constitutional isomer of 3′-Sialyllactose, which contains the same monosaccharide moieties, with the linkage between Neu5Ac and D-galactose being α-(2-3) instead of α-(2-6).

6′-SL sodium salt is characterised by the molecular formula: $C_{23}H_{38}NO_{19}Na$; molecular mass: 655.53 Da; CAS No 157574-76-0; IUPAC name: N-Acetyl-α-D-neuraminyl-(2→6)-β-D-galactopyranosyl-(1→4)-D-glucose, sodium salt

The structure has been confirmed by monodimensional (1D) and two-dimensional (2D) ^{1}H and ^{13}C Nuclear Magnetic Resonance spectroscopy (NMR). ^{1}H NMR spectra show chemical shifts fully corresponding to those reported in the literature for 6′-SL (Urashima et al., 2018), together with a minor impurity (i.e. lactose). By Nuclear Overhauser Effect Spectroscopy (NOESY), some intra- and inter-residual correlations were shown. All correlations for carbons involved in glycosidic linkages were evidenced in the Hetero Single Quantum Coherence (HSQC) spectrum and the interglycosidic correlations showing the positions of the glycosidic linkages were confirmed by the Heteronuclear Multiple Bond Correlation (HMBC) spectrum. Taken together, NMR data confirm that the glycosidic linkage between N-acetylneuraminic acid (Neu5Ac) C2 and the adjacent galactose (Gal H6 and H6′) is α-(2-6). An inter-residual correlation was also observed between Gal H1 and Glc C4 in the HMBC spectrum, this together with the coupling constants of the Gal H1 and Glc H1 signals are indicating the

1-4 connection between the Gal and Glc units and that the pyranose configuration is β for the Gal unit and that the reducing end glucose is in equilibrium between the α- and the β-anomeric forms. Also, the mass fragmentation pattern of 6′-SL in MS/MS spectrometry has been provided and is consistent with the structure proposed (Figure 1, from the dossier).

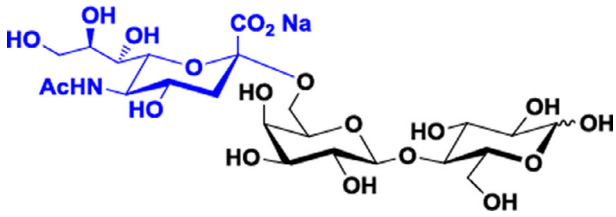

Figure 1: Structure of 6′-SL sodium salt

The 6′-SL produced by the microbial fermentation described has been shown to be chemically and structurally identical to its naturally occurring counterpart present in human milk oligosaccharides by mono- and two-dimensional NMR and is therefore considered as HiMO.

3.3. Production process

According to the data provided by the applicant, the NF is produced pursuant to Good Manufacturing Practice (GMP) and Hazard Analysis Critical Control Points (HACCP) principles. The production process is comparable to those of the already authorised HiMOs, such as LNnT (lacto-*N*-neotetraose), 2′-FL (2′-Fucosyllactose), 2′-FL/DFL (difucosyllactose) and LNT (lacto-*N*-tetraose) also previously assessed by EFSA (2′FL/DFL and LNT, EFSA NDA Panel, 2019a,b).

The manufacturing process can be broadly divided into two stages. In the first stage, D-lactose and D-glucose are used for production of 6′-SL by the adapted cellular metabolism of the producing microorganism, which uses D-glucose as an energy source and D-lactose as a substrate for 6′-SL biosynthesis. At the end of the fermentation process, the production microorganism is entirely removed from the medium by filtration. The second stage of the process includes several purification steps after which the acidic 6′-SL solution is alkalised with NaOH with formation of the 6′-SL sodium salt to obtain the NF as a dried powdered mixture.

The microorganism used in the fermentation process for the production of the NF is a genetically modified derivative of the parental strain *E. coli* K-12 DH1 (F-ʎ-gyrA96 recA1 relA1 endA1 thi-1 hsdR17 supE44), that was obtained by the applicant from the German Collection of Microorganisms and Cell cultures (DSMZ) and deposited under DSM No. 4235. The derivative producing strain has been deposited in the DSMZ in Braunschweig, Germany.

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 of other derivative strains, including *E. coli* K-12 DH1, were sequenced and compared with other *E. coli* strains. The results indicate that *E. coli* K-12 and its derivatives show genomic differences compared to pathogenic strains (Blattner et al., 1997; Lukjancenko et al., 2010). Although the species *E. coli* was considered not suitable for qualified presumption of safety (QPS) status (EFSA BIOHAZ Panel, 2018), *E. coli* K-12 is considered as a safe and non-pathogenic or toxigenic microorganism widely used for biotechnological applications (Gorbach, 1978; OECD 1986; Muhldorfer and Hacker, 1994; U.S. EPA, 1997).

The applicant provided a detailed description of the genetic modification steps applied to the parental strain *E. coli* K-12 DH1 to obtain the derivative strain used to produce the NF.

The absence of viable cells of the producing microorganism in the NF was demonstrated by testing five batches of the NF for bacteria from the *Enterobacteriaceae* family according to internationally recognised methods (ISO 21528-1:2004, MSZ ISO 21528-2:2007). The specification for *Enterobacteriaceae* set at 'absent in 10 g' ensures absence in the final product of the microorganism, since *E. coli* belongs to the *Enterobacteriaceae* family. Furthermore, the absence of residual proteins is confirmed by an adaptation of the Bradford protein method, which has been developed to quantify proteins down to 0.005 w/w% level. No residual protein was detected in the final method above the 0.01 w/w% specification.

In addition, no residual DNA from the production organism was detected in five independent batches of the NF using three quantitative polymerase chain reaction (PCR) assays targeting short subsequences of specific inserted genes as well as a short subsequence of the 23S rRNA subunit of

E. coli. Upon EFSA's request, the applicant provided data to demonstrate the validity of the method in accordance to the EFSA guidance on microorganisms used 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

The NF contains 6′-SL sodium salt as the primary ingredient (around 95% w/w dry matter as sodium salt). The remainder is a mixture of substances including D-lactose, sialic acid, 6′-sialyl-lactulose and other carbohydrates.

The Panel therefore notes that although the main component of the NF is 6′-SL sodium salt, other fractions (lactose, sialic acid and 6′-sialyl-lactulose) are present in different amounts in the NF. Lactose is the most prevalent molecule in human breast milk (approximately 7 g/100 mL). Sialic acid is an endogenous human and ubiquitous nutritional monosaccharide (EFSA NDA Panel, 2017; Röhrig et al., 2017) while 6′-sialyl-lactulose is derived from 6′-SL by isomerisation of the terminal glucose moiety into fructose mainly under alkaline conditions during the production process (Zeng et al., 2018). In representative batches of the NF, the amount of lactose ranged between 2% and 3% while the amount of each of the other two saccharides was below 1%.

With regard to the physicochemical properties, the NF can be described as white to off-white amorphous powder or agglomerate. It is readily soluble in aqueous solutions (max. 500 mg/mL, 25°C).

The applicant provided results of batch-to-batch compositional analyses for five batches of the NF (Table 1). 6′-SL and minor impurities have been analysed by high performance anion exchange chromatography coupled to pulsed amperometric detection (HPAEC-PAD), using 'in-house' validated methods as well as analysis by certified external international laboratories.

The microbiological purity of batches of the NF has been assessed for non-pathogenic microorganisms (bacteria, yeasts and moulds) as general hygiene indicators, as well as for selected food-borne pathogens.

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

Table 1: Batch-to-batch analysis for five batches of NF

Parameters	Batches					Mean ± SD
	CPN5315 1000117 FD	CPN5315 1000317 FD	CPN5315 1000417 FD	CPN5315 1000617 FD	CPN5315 1000717 FD	
Physicochemical properties						
pH (20°C, 5% solution)	5.4	5.2	5.3	5.6	5.8	5.5 ± 0.2
Composition						
HIMS (w/w % dry matter)[(a)]	98.0	99.9	99.4	96.6	98.1	98.4 ± 1.3
6′-SL sodium salt (w/w % dry matter)	94.3	96.8	97.1	93.9	95.3	95.5 ± 1.4
D-Lactose [w/w %]	2.89	2.52	2.01	1.92	1.94	2.26 ± 0.43
Sialic acid [w/w %]	0.68	0.51	0.33	0.81	0.85	0.64 ± 0.22
6′-sialyl-lactulose [w/w %]	0.54	0.75	0.76	0.65	0.72	0.68 ± 0.09
Sum of other carbohydrates [w/w %]	0.56	0.55	0.42	0.63	0.66	0.56 ± 0.08
Total carbohydrates (dry matter) [w/w %][(b)]	99.0	101.1	100.6	97.9	99.5	99.6 ± 1.3
Water [w/w %]	0.38	0.44	0.42	0.44	0.47	0.43 ± 0.03
Sodium [w/w %]	3.27	3.23	3.06	3.15	3.25	3.19 ± 0.09

Parameters	Batches					Mean ± SD
	CPN5315 1000117 FD	CPN5315 1000317 FD	CPN5315 1000417 FD	CPN5315 1000617 FD	CPN5315 1000717 FD	
Chloride by IC [w/w %]	0.130	< 0.003	< 0.002	0.049	0.079	0.053 ± 0.048
Residual proteins (Bradford method) [w/w %]	< LoR	< LoR	< LoR	< LoR	0.0051	
Microbiological parameters						
Aerobic mesophilic total plate count [CFU/g]	< 10	< 10	< 10	< 10	< 10	< 10
Enterobacteriaceae [in 10 g]	Absent	Absent	Absent	Absent	Absent	Absent
Salmonella spp. [in 25 g]	Absent	Absent	Absent	Absent	Absent	Absent
Cronobacter (Enterobacter) sakazakii [in 10 g]	Absent	Absent	Absent	Absent	Absent	Absent
Listeria monocytogenes [in 25 g]	Absent	Absent	Absent	Absent	Absent	Absent
Bacillus cereus [CFU/g]	< 10	< 10	< 10	< 10	< 10	< 10
Yeasts [CFU/g]	< 10	< 10	< 10	< 10	< 10	< 10
Moulds [CFU/g]	< 10	< 10	< 10	< 10	< 10	< 10
Residual endotoxins [EU/mg]	0.014	NA	0.340	0.100	0.840	0.32 ± 0.32

HiMS: human identical milk saccharides, it includes 6′-SL sodium salt, D-lactose and sialic acid; CFU: colony forming units; 6′-SL: 6′-Sialyllactose; IC: ion chromatography; ± SD: standard deviation; LoR (limit of reporting) 0.0050 w/w %; EU: endotoxin units; NA: not available.
(a): Sum of 6′-SL sodium salt, lactose and sialic acid.
(b): Sum of all carbohydrates.

3.4.1. Stability

Stability of NF

The bulk stability of the powdered NF produced by fermentation has been investigated in two representative batches, in an ongoing 5-year study under real-time conditions (25°C, 60% relative humidity (RH)) and an ongoing 2-year study under accelerated conditions (40°C, 75% RH). Results were provided for both studies for 12 months.

Upon EFSA's request for additional information, the applicant provided interim results of the same two batches of the NF up to 24 months for both the real-time stability and accelerated stability studies. Results on composition, sensory testing and microbiological parameters indicate that there is no appreciable degradation of NF ingredients, no changes in impurity profile and no alterations in the microbiological quality of the NF.

On the basis of these data and of the accelerated stability studies at 40°C, the reaction rate of isomerisation was calculated and extrapolated by the Arrhenius analysis to 25°C (which is the temperature of storage of the NF), indicating, according to the applicant, a shelf-life of 5 years for the NF.

In addition, results for stress stability studies for the NF have been provided by the applicant. The NF in the solid state was heated in an oven at 80°C in ambient humidity for 28 days. The results show that 6′-SL decreased (from 82.9% to 75.3%), lactose and sialic acid slightly increased, while 6′-Sialyl-lactulose, not detected at time 0, increased up to 9.5%. At higher humidity, 6′-SL remained steady for the first 2 days, then slightly decreased, while 6′-sialyl-lactulose increased up to 11.4% at the end of the month.

Based on the data available, the Panel considers that the NF is expected to be stable for at least 24 months when stored at room temperature and low humidity in the solid state.

Stability of NF under the intended conditions of use

The NF was stable for a month in water solutions at neutral pH and 35°C while in acidic conditions (pH 3 at 35°C), it was almost completely hydrolysed to lactose and sialic acid. Under alkaline conditions (pH 9 at 35°C for a month), isomerisation of 6′-SL to 6′-sialyl-lactulose was observed (up to approximately 13%). Both degradation pathways were observed on amorphous 6′-SL freeze-dried from solutions with different pHs by forced stability studies (at 60°C for 2 months and at 80°C for 1 month). The 6′-SL was most stable when obtained from solutions at pH 5, where no significant hydrolysis was observed.

When oxidative stress was applied to a 6′-SL solution for one day, no degradation was observed neither in the presence of 0.1% H_2O_2 nor in the presence of 0.1 M equivalent of ACVA (4,4′-azobis (cyanovaleric acid)).

The stability of the NF in the powdered IF has been investigated following 12 months storage at different temperatures (4–37°C) and the target concentration of 0.37 g/100 g (dry matter) was found to be constant over time.

No other stability data for the NF in real food matrices have been provided. However, the applicant made reference to studies conducted with other HiMS (human identical milk saccharides) (i.e. 2′-FL, LNnT and sialic acid) in food matrices (e.g. yoghurt, ready-to-drink flavoured milk) where no significant loss of the added HiMO has been observed. In its previous assessment for these HiMOs, the NDA Panel concluded that 'the data provide sufficient information with respect to the stability of the NF' (EFSA NDA Panel, 2015, 2017).

The Panel considers that the available data provide sufficient information with respect to the stability of the NF in the food matrices at neutral pH, when stored at room temperature under proper storage conditions.

3.5. Specifications

The specifications of the NF as proposed by the applicant are presented in Table 2. The parameters include the main components of the NF mixture of HiMS, predominantly 6′-SL sodium salt, D-lactose, sialic acid and the isomer 6′-sialyl-lactulose. The sum of relevant HiMSs has been introduced by the applicant as a parameter to ensure a highly consistent product quality. The 6′-SL sodium salt content is specifically set at a minimum of 90 w/w % (dry matter).

Another parameter called 'sum of other carbohydrates' has been introduced, to include other carbohydrates individually present in low concentrations, up to a total amount of 3% w/w.

Microbiological parameters for *Listeria monocytogenes, Cronobacter (Enterobacter) sakazakii* and *Bacillus cereus* are monitored using internal specifications.

Analyses were performed using internationally recognised methods or newly developed and validated analytical protocols at Glycom's Research & Development Department and confirmed by accredited laboratories.

The Panel considers that the information provided on the specification of the NF is sufficient.

Table 2: Specifications of the NF

Description: 6′-Sialyllactose sodium salt (6′-SL sodium salt) is a purified, white to off-white powder or agglomerates that is produced by a microbial process and contains limited levels of lactose and sialic acid		
Source: A genetically modified strain of *Escherichia coli* K-12 DH1 MDO		
Parameter	**Specification**	**Method**
Sum of HiMS[(a)] (w/w % dry matter)	≥ 94.0	HPAEC/PAD
6′-SL sodium salt (w/w % dry matter)	≥ 90.0	HPAEC/PAD
D-Lactose [w/w %]	≤ 5.0	HPAEC/PAD
Sialic acid [w/w %]	≤ 2.0	HPAEC/PAD
6′-sialyl-lactulose [w/w %]	≤ 3.0	HPAEC/PAD
Sum of other carbohydrates [w/w %]	≤ 3.0	HPAEC/PAD
pH (20°C, 5% solution)	4.5–6.0	Eur. Ph. 9.2 2.2.3 (07/2016:20203)
Water [w/w %]	≤ 6.0	Karl-Fisher

Sodium [w/w %]	2.5–4.5	EN 13805:2002; EPA-6010C:2007
Chloride by IC [w/w %]	≤ 1.0	ISO10304-2:1999
Residual protein [w/w %]	≤ 0.01	Bradford assay (UV spectroscopy)(b)
Microbiological Parameters		
Aerobic mesophilic total plate count	≤ 1,000 CFU/g	ISO 4833-1:2014
Enterobacteriaceae	≤ 10 CFU/g	ISO 21528-1:2004, ISO 21528-2:2007
Salmonella spp.	Absent in 25 g	ISO 6579:2006
Yeasts	≤ 100 CFU/g	ISO 21527-2:2008
Moulds	≤ 100 CFU/g	ISO 21527-2:2008
Residual endotoxins	≤ 10 EU/mg	Eur. Ph. 2.6.14

6′-SL sodium salt: 6′-Sialyllactose sodium salt; CFU: colony-forming units; EU: endotoxin units; Eur. Ph.: European Pharmacopoeia; HiMO: human-identical milk oligosaccharides; HPLC: high-performance liquid chromatography; IC: ion chromatography; CAD: charged aerosol detection; PAD: pulsed amperometric detection; HPAEC: high performance anion exchange chromatography; KF: Karl-Fischer; RT: retention time; ISO: international organization for standardization; UV: ultraviolet.
(a): Sum of HiMS: 6′-SL sodium salt, D-lactose and sialic acid.
(b): LOR (limit of reporting) = 17 mg/kg.

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

3.6.1. History of use of the NF

The NF does not have a history of use.

6′-SL has been detected in domestic farm animal milk, albeit at lower concentrations as compared to human milk. Oligosaccharides in bovine milk are 20 times less concentrated than in human milk; however, sialylated oligosaccharides accounted for approximately up to 80% of the total oligosaccharide pools. The amount of 6′-SL in bovine milk is estimated to be ranging from 4 to 10 mg/L and up to 100 mg/L in colostrum (Aldredge et al., 2013; Urashima et al., 2013; Albrecht et al., 2014), more than 50 times lower than human breast milk levels.

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

Human breast milk contains a family of structurally related oligosaccharides, known as HMOs and representing the third largest fraction of solid components. The highest concentrations of HMOs occur in human colostrum (20–25 g/L), and concentrations between 5 and 20 g/L occur in mature human milk (Thurl et al., 2010; Bode, 2012; Urashima et al., 2018). HMOs' concentration and composition vary across mothers and over the course of lactation. 6′-SL belongs to the subfraction of 'acidic' HMOs, which is characterised by the presence of sialic acids, and the whole subfraction accounts for 1.5–3.3 g/L (Thurl et al., 2010; Rijnierse et al., 2011; Bode, 2012). 6′-SL is the predominant acidic HMO and one of the most abundant HMOs along with 2′-FL, lacto-N-fucopentaose I, LNT and LNnT.

There are two naturally occurring sialyllactoses, which are constitutional isomers with a minimal structural difference: the oligosaccharide backbone can be sialylated by α-(2-3) or α-(2-6) linkages, resulting in 3′-SL or 6′-SL, respectively. The two forms have been shown to have similar functions and biological roles (Tarr et al., 2015).

Several publications on HMOs and 6′-SL in human milk have been provided by the applicant.

The highest concentration of 6′-SL and other HMOs in human milk is reported in colostrum, and the concentration is depending on the stage of lactation (Asakuma et al., 2008; Austin et al., 2016; Coppa et al., 1999, 2011; Kunz et al., 2017; McGuire et al., 2017; Spevacek et al., 2015; Thurl et al., 2010, 2017). Thurl et al. (2017) summarised the findings from 21 studies and reported that the content of 6′-SL in milk from mothers who delivered at term ranged from 0.38 to 0.91 g/L (average 0.64 g/L). It was noted that the range of 6′-SL was slightly wider (ranging from 0.25 to 1.08 g/L, average 0.66 g/L) in mothers who delivered preterm.

Based on the mean and the highest reported occurrence levels of 6′-SL in human milk from mothers who had a preterm delivery, as reported by Thurl et al. (2017), and considering the average and high daily intake of breast milk (800 mL and 1,200 mL, respectively) for infants from 0 to 6 months (EFSA NDA Panel, 2013), the daily intake levels of 6′-SL from human milk for a 6.7 kg body weight (bw) infant (EFSA Scientific Committee, 2012) have been calculated (Table 3). This default

body weight used by the NDA Panel is for an infant of 3–6 months of age, who is more likely than younger infants to consume these volumes of human milk.

Table 3: Estimated daily intake levels of 6′-SL from human milk (800 and 1,200 mL) for infants of 6.7 kg bw, based on mean and high concentration of 0.66 and 1.08 g/L, respectively, of 6′-SL measured in human milk from mothers who had a preterm delivery (Thurl et al., 2017)

	Daily intake levels (mg/kg bw) from 800 mL of human milk		Daily intake levels (mg/kg bw) from 1,200 mL of human milk	
	Mean concentration	High concentration	Mean concentration	High concentration
6′-SL	79	129	118	193

bw: body weight.

3.7. Proposed uses and use levels and anticipated intake

3.7.1. Target population

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

3.7.2. Proposed uses and use levels

The NF is intended to be added to a variety of foods, at the maximum use levels as indicated in Table 4. The Panel notes that for the category 'Food for special medical purposes', the applicant did not propose maximum use levels or maximum intake levels.

The applicant also intends to market the NF as a food supplement, at the maximum daily intake of 1 g for individuals above 3 years of age or at a maximum level of 0.4 or 0.3 g/day when intended for infants (up to 11 months) or young children (12–35 months), respectively.

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

Table 4: Proposed food uses and maximum use levels of the 6′-SL

EU food category number	Food category name	Proposed Maximum use level
1	**Dairy products and analogues**	
1.1	Unflavoured pasteurised and unflavoured sterilised (including UHT) milk	0.5 g/L
1.2/1.3	Unflavoured fermented milk-based products	0.5 g/L beverages
		2.5 g/kg for products other than beverages
1.4	Flavoured fermented milk-based products including heat-treated products	0.5 g/L beverages
		5 g/kg for products other than beverages
7	**Bakery wares**	
7.2	Fine bakery wares. Cereal bars only	5 g/kg
13	**Foods for Special Groups (FSG)**	
13.1	**Foods for infants and young children**	
13.1.1	Infant formula as defined in Regulation (EU) No 609/2013	0.4 g/L in the final product ready for use, marketed as such or reconstituted as instructed by the manufacturer
13.1.2	Follow-on formula as defined in Regulation (EU) No 609/2013	0.3 g/L in the final product ready for use, marketed as such or reconstituted as instructed by the manufacturer
13.1.3	Processed cereal-based food and baby food for infants and young children as defined in Regulation (EU) No 609/2013	0.3 g/L in the final product ready for use, marketed as such or reconstituted as instructed by the manufacturer
		2.5 g/kg for products other than beverages

EU food category number	Food category name	Proposed Maximum use level
13.1.4	Milk-based drinks and similar products intended for young children	0.3 g/L in the final product ready for use, marketed as such or reconstituted as instructed by the manufacturer
		2.5 g/kg for products other than beverages
13.2	**Foods for special medical purposes as defined in Regulation (EU) No 609/2013**	
13.2	Foods for special medical purposes as defined in Regulation (EU) No 609/2013	On case-by-case basis
13.3	**Total diet replacement for weight control as defined in Regulation (EU) No 609/2013**	
13.3	Total diet replacement for weight control as defined in Regulation (EU) No 609/2013	1.0 g/L beverages 10 g/kg for products other than beverages
14	**Beverages**	
14.1.4	Flavoured drinks (excluding cola-type drinks)	0.5 g/L

UHT: ultra-high temperature.

3.7.3. Anticipated intake of the NF

Anticipated intake of the NF from the consumption of infant formula in infants up to 16 weeks of age

IF is expected to be the only food consumed by infants aged 0–16 weeks who are not breastfed. A high consumption of IF has been estimated to be 260 mL/kg bw per day for infants aged 0–16 weeks (EFSA Scientific Committee, 2017). Based on the maximum proposed use level of the NF (0.4 g/L in IF), the high intake of the NF from IF alone is estimated for an infant of 6.7 kg to be 104 mg/kg bw per day.

The Panel notes that the anticipated daily intake of the NF from the consumption of IF (only) does not exceed the estimated high daily intake of 6′-SL in breast-fed infants per kg/bw (Table 3).

Anticipated intake of 6′-SL from the proposed uses and use levels of the NF

The applicant estimated the daily intake of the NF by using the EFSA Food Additive Intake model (FAIM) tool (FAIM 2.0, 2017) and compared the intake to surveys conducted in the UK (NDNS (National Diet and Nutrition Survey) and its complement DNSIYC (Diet and Nutrition Survey of Infants and Young Children)). However, considering that the food categories in the FAIM tool, which are based on Regulation (EC) No 1333/2008[3], do not allow a precise matching with the food categories proposed for the NF, EFSA performed an assessment of the anticipated daily intake of the NF, at the maximum proposed use levels of the NF, using individual data from EU dietary surveys (EFSA, 2011) and by applying the FoodEx2 classification (Table 5). The range of mean and 95th percentile anticipated daily intake of the NF for all subjects, among the EU dietary surveys, is presented in Table 6.

The refined anticipated 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 (it can be found in the online version of this output under 'Supporting information': https://doi.org/10.2903/j.efsa.2020.6097).

[3] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives (Text with EEA relevance) OJ L 354, 31.12.2008, p. 16–33.

Table 5: FoodEx2 categories and maximum use levels of the NF used in the refined estimate of the anticipated daily intake of the NF using individual data from EU dietary surveys

CODE	FoodEx2 level	Food category	Maximum use level of the NF mg/100 g
A02LV	5	Cow milk	50
A0CXA	5	European buffalo milk	50
A02MC	5	Sheep milk	50
A02MB	4	Goat milk	50
A02MV	3	Butter milk	50
A02NQ	4	Yoghurt drinks	50
A02NR	4	Probiotic milk-like drinks	50
A02NV	5	Kefir	50
A02NE	4	Yoghurt	250
A00EY	4	Cereal bars	500
A00EZ	4	Cereal bars plain	500
A00FA	4	Cereal bars mixed	500
A03PZ	4	Infant formulae, powder	325
A03QE	4	Infant formulae, liquid	40
A03QK	4	Follow-on formulae, powder	242.5
A03QQ	4	Follow-on formulae, liquid	30
A03QZ	3	Cereals with an added high protein food which have to be reconstituted	150
A03QY	3	Simple cereals which have to be reconstituted	150
A0BZF	3	Cereals with added high protein food reconstituted	30
A0BZE	3	Simple cereals for infants and children reconstituted	30
A03RA	3	Biscuits, rusks and cookies for children	250
A03RC	2	Ready-to-eat meal for infants and young children	250
A03RB	3	Pasta for children	250
A03RN	3	Fruit and vegetable juices and nectars specific for infants and young children	30
A03RP	3	Special food for children's growth	30
A03RT	4	Total daily diet replacement for weight reduction	1,000
A0EQN	5	Soft drinks with minor amounts of fruits or flavours	50
A03EA	5	Soft drink with fruit juice (fruit content below the minimum for nectars)	50
A03EX	5	Soft drink, flavoured, no fruit	50

Table 6: Ranges among EU surveys of the estimated daily intake of the NF (mg/kg bw), based on the individual food intake data from the EFSA Comprehensive Food Consumption Database

Age groups	Number of EU dietary surveys	Estimated daily intake of the NF – all subjects (mg/kg bw)	
		Range of means (lowest and highest) among EU dietary surveys	Range of 95th percentile (lowest and highest) among EU dietary surveys
Infants (up to 11 months)	11	16–74	38–192
Young children or toddlers (12–35 months)	14	15–46	39–147
Other children (3–9 years)	19	6–25	16–61
Adolescents (10–17 years)	18	3–8	7–19

Age groups	Number of EU dietary surveys	Estimated daily intake of the NF – all subjects (mg/kg bw)	
		Range of means (lowest and highest) among EU dietary surveys	Range of 95th percentile (lowest and highest) among EU dietary surveys
Adults (18–64 years)	19	1–5	5–13
Elderly (≥ 65 years)	18	1–4	5–10
Pregnant women	2	1–5	4–11
Lactating women	2	4-5	10–11

bw: body weight.

The Panel notes that the highest estimated 95th percentile intake (i.e. 192 mg/kg bw) on the basis of 11 dietary surveys covered by the EFSA Food Consumption Database is similar to the high estimate for 6′-SL from human milk (i.e. 193 mg/kg bw, Table 3) in infants.

Considering the conservative assumption underlying this type of intake assessment, in particular, assuming that all foods of the proposed food categories consumed by infants contain the NF at the maximum proposed use levels, the Panel considers very unlikely that even the most exposed population category (infants up to 11 months) would exceed the high intake level estimated for 6′-SL intake from human milk.

Anticipated intake of 6′-SL from food supplements

The applicant has proposed a maximum daily intake of 1 g of the NF as food supplements for individuals above 3 years of age or at a maximum level of 0.4 or 0.3 g/day when intended for infants (up to 11 months) or young children (12–35 months), respectively. Food supplements are not intended to be used if other foods with added 6′-SL sodium salt are consumed on the same day. For infants and young children, food supplements are not intended to be used if breast milk or other foods with added NF are consumed on the same day.

The maximum daily intake from food supplements of the NF (i.e. 1 g/day) results in a maximum daily intake ranging from 14 to 43 mg/kg bw in the general population. The maximum dose of 0.4 g/day in infants (bw of 6.7 kg) and 0.3 g/day in young children (bw of 11.9 kg) results in a maximum intake of 60 or 25 mg/kg bw, respectively (default body weight values from EFSA Scientific Committee (2012)).

The Panel notes that the maximum daily intake of 6′-SL from the use of NF as food supplement (i.e. from 300 mg to 1 g/day) for any population category does not exceed the estimated high daily intake of 6′-SL from human milk calculated for infants on a body weight basis (Table 3).

3.7.4. Combined intake from the NF and other sources

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

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

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

There are no data submitted for the NF.

HMOs, including 6′-SL, are considered 'non-digestible oligosaccharides' (EFSA NDA Panel, 2014) since they do not undergo any significant digestion in the upper gastrointestinal tract (Brand-Miller et al., 1995, 1998; Engfer et al., 2000; Gnoth et al., 2000; Chaturvedi et al., 2001; Rudloff and Kunz, 2012).

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

In a paper from Goehring et al. (2014), concentrations of 6′-SL were measured in breast milk and plasma and urine of breastfed infants. Concentrations in breast milk measured were within the

expected range (mean of 0.72 g/L) and urinary excretion was accounting for approximately 4% of the intake from breast milk. However, there was no clear correlation between urinary and milk concentrations and the analytical method used was unable to detect 6′-SL in plasma.

It has been observed that an infant, who is breastfed, may receive up to 150 mg of individual oligosaccharides from each feed and that an amount up to 3 mg per day is found in the urine (Rudloff and Kunz, 2012; Rudloff et al., 2012). Specifically, for 6′-SL (and few other HMOs), it has been demonstrated that absorption and urinary excretion are also occurring in the rat (Vazquez et al., 2017).

Based on information available on HMOs, the Panel considers that limited digestion of the NF occurs in the upper gastrointestinal tract and that only small amounts are expected to be absorbed. Moreover, there are no indications that the absorption of 6′-SL or other components of the NF may differ from that of similar components in human milk.

3.9. Nutritional information

The NF is mainly composed of the non-digestible oligosaccharide 6′-SL (EFSA NDA Panel, 2014).

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

The Panel notes that the NF, being a sodium salt, may contribute to the daily sodium intake.

In its Opinion on DRVs for sodium, the NDA Panel has provided advice on levels of sodium intake that are considered safe and adequate[4] for population groups aged 1 year and older (EFSA NDA Panel, 2019c). Considering the maximum sodium content in the NF of 4.5%, the intake of sodium from the NF is expected to represent up to 7% (up to 79 mg sodium/day) of the sodium intake of 1.1 g/day considered as safe and adequate for toddlers (1–3 years). For other children and adults, the intake of sodium from the NF can represent up to 5% of the sodium intake levels considered as safe and adequate for these age groups.

As for infants up to the age of 6 months consuming IF, the maximum sodium intake from the NF would be approximately 31 mg per day considering a daily intake of IF of 260 mL/kg. This corresponds to about 25% of the daily sodium intake of exclusively breast-fed infants (120 mg sodium/day during the first 6 months; EFSA NDA Panel, 2019c).

For older infants aged 7–11 months, the Panel established an adequate intake (AI)[5] of 200 mg/day (EFSA NDA Panel, 2019c). In this age group, the maximum sodium intake from the NF is estimated to be 76 mg sodium/day, which corresponds to 38% of the AI.

3.10. Toxicological information

The list of toxicological studies, which were provided and claimed proprietary by the applicant, is reported in Table 7. These studies were conducted with the NF (batch CPN53151000317 FD), which was constituted by 96.8% w/w dry matter of 6′-SL sodium salt (with a total of 99.9% dry matter of HiMSs).

The applicant also provided information on toxicological studies conducted with enzymatically produced 6′-SL (Gurung et al., 2018) and the Panel considers that these toxicological studies can provide supporting evidence for the safety assessment of the NF (see Section 3.10.3). The Panel noted that toxicological studies were also conducted with 3′-SL, the constitutional isomer of 6′-SL, which was obtained by microbial fermentation using genetically modified strains of *E. coli* K-12.

[4] These levels of sodium intake are called 'safe' because it takes account of the evidence describing the relationship between sodium intake and CVD risk in the general population and 'adequate' in line with the definition of an Adequate Intake.

[5] An Adequate Intake (AI) is the value set when there is not enough data to calculate an average requirement for a nutrient. An AI is the average level of intake of a nutrient, based on observations or experiments that is assumed to be adequate.

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

Test material	Reference	Type of study
NF (96.8% w/w dry matter 6′-SL sodium salt)	Unpublished study report (2018a)	Bacterial reverse mutation test (Ames test)
	Unpublished study report (2018b)	*In vitro* mammalian Cell Micronucleus Test
	Unpublished study report (2018c)	14-day DRF repeated dose oral toxicity study in neonatal rats
	Unpublished study report (2018d)	90-day GLP repeated dose oral toxicity study in neonatal rats

DRF: dose range finding.

The Panel also noted that under acidic conditions, the NF will be hydrolysed to lactose and sialic acid. The amount of sialic acid potentially formed (at the maximum level and intake, i.e. for infants at 95th percentile (Table 6), would result in 3.8 mg/kg bw), which is lower than the intake based on natural levels in human milk (that is 11 mg/kg bw (EFSA NDA Panel, 2017)). Under alkaline conditions, 6′-sialyl-lactulose would also be formed although the content would remain very low (up to 5.8 mg/kg bw) and is considered not having any laxative effect (suggested use in infants to treat constipation is 2.5 mL syrup/day corresponding to approximately 1.65 g lactulose/day).

3.10.1. Genotoxicity

The potential genotoxicity of the NF was investigated in a bacterial reverse mutation test and an *in vitro* mammalian cell micronucleus test (Unpublished study report, 2018a). These studies were conducted in compliance with Organisation for Economic Co-operation and Development (OECD) principles of Good Laboratory Practice (GLP) (OECD, 1998a) and in accordance with the OECD test guidelines No 471 and 487 of 1997 and 2016, respectively.

The *in vitro* assessment of the mutagenic potential of the NF was performed with histidine-dependent auxotrophic mutants of *Salmonella typhimurium*, strains TA1535, TA1537, TA98 and TA100, and a tryptophan-dependent mutant of *Escherichia coli*, strain WP2 uvrA (pKM101), that were exposed to the NF diluted in water at concentrations up to 5,000 μg/plate either in the presence or absence of liver microsomal fractions (S9). No substantial, reproducible or dose-related increases in revertant colony numbers over control counts were observed with any of the strains following exposure to the NF at any concentration (irrespective of the presence or absence of S9). No evidence of toxicity was obtained following exposure to the NF. Therefore, the NF was shown to be non-mutagenic at concentrations up to 5,000 μg/plate, in the absence or presence of metabolic activation.

In the *in vitro* mammalian cell micronucleus test, concentrations of 6′-SL sodium salt up to 2,000 μg/mL were tested to assess the potential of the NF to cause an increase in the induction of micronuclei in *in vitro* cultured human peripheral blood lymphocytes in the presence or absence of metabolic activation (S9 fraction). No statistically significant increases in the number of binucleate cells containing micronuclei both after 3-h treatment in the presence of S9 mix or following 20-h treatment in the absence of S9 were recorded. The NF did not show any evidence of clastogenicity or aneugenicity in the absence and presence of metabolic activation.

Based on the results of these studies, the Panel considers that there are no concerns regarding genotoxicity of the NF.

3.10.2. Repeated dose toxicity studies

The applicant provided a 14-day repeated dose oral toxicity study where groups of eight Crl:CD(SD) neonatal rats/sex were given water (control), 4,000 and 5,000 mg/kg bw per day of NF expressed as 6′-SL sodium salt by oral gavage starting from day 7 of age (Unpublished study report, 2018c). There were no deaths or any variations in clinical signs, body weight or macroscopic pathology attributable to the NF. Clinical pathology parameters were not assessed.

In a 90-day study, groups of 10 Crl:CD(SD) neonatal rats/sex were given water (control), 1,000, 3,000 or 5,000 mg/kg bw per day of NF expressed as 6′-SL sodium salt by oral gavage starting from 7 days of age once daily for at least 90 days, until the day before necropsy (Unpublished study report, 2018d). An additional control group was treated with oligofructose powder (a non-digestible oligosaccharide permitted in infant nutrition) at 5,000 mg/kg per day, to compare any effects related

to the general fibre-like characteristics at the same high dose. Five additional rats/sex for control groups and high dose of NF were observed over a 4-week recovery period.

This study has been designed based upon the OECD TG408 (OECD, 1998b), but has been adapted (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). There were no test item-related deaths or any variations in clinical signs attributable to the NF.

Statistically significant changes were observed in several parameters (summary results can be found in the online version of this output under 'Supporting information': https://doi.org/10.2903/j.efsa.2020.6097). However, the findings observed were of low magnitude, often without dose correlation, sometimes occurring only in one gender or at the end of recovery period and are considered by the Panel as overall not biologically relevant. The Panel also notes that this specific experimental design, with treatment of animals by gavage prior to weaning, may result in high variability in several parameters.

At the microscopic examination, four rats receiving the highest dose showed small and soft testis characterised by severe unilateral tubular atrophy and absence of sperm in the epididymis. Three of the four rats affected were from the same litter and only one testis was affected in each animal. There was no clear explanation for the observation. In the absence of such explanation, the author of the study considered the intermediate dose of 3,000 mg/kg bw per day of the 6′-SL sodium salt as the NOAEL (No Observed Adverse Effect Level). The Panel agrees with this conclusion.

3.10.3. Toxicological studies conducted with 6′-SL enzymatically synthetised

The applicant reported that in studies conducted following 'Toxicological Principles for the Safety Assessment of Food Ingredients' (FDA 'Redbook 2000') and in accordance with GLP principles, 6′-SL enzymatically produced (purity > 98%) did not elicit adverse effects in any of the tested doses or concentrations. No signs of mutagenicity, clastogenicity or aneugenicity in genotoxicity studies (bacterial mutation assay, chromosomal aberrations assay and *in vivo* micronucleus) were noted. In the 90-day oral study in the rat (weaned rats of standard age), the highest dose of 5,000 mg/kg bw per day was identified as an NOAEL. The authors concluded that 'The 6′-SL sodium salt showed toxicity profiles comparable to other carbohydrates and HMOs' (Gurung et al., 2018).

Table 8: List of toxicological studies conducted with 6′-SL produced by enzymatic synthesis

Test material	Reference	Type of study	Experimental details
6′-SL (> 98%)	Gurung et al. (2018)	Bacterial Reverse Mutation Test	100, 300, 625, 1,250, 2,500 and 5,000 μg 6′-SL plate; ± S9
		In vitro chromosome aberration test (CHL)	225, 450, and 900 μg 6′-SL/mL; ± S9
		In vivo mammalian Cell Micronucleus Test	500, 1,000 and 2,000 mg 6′-SL/kg bw
		Acute Toxicity Study in the SD Rat	5 SD rats/sex per group; 5,000, 10,000, 15,000 and 20,000 mg 6′-SL/kg bw per day
		90-day GLP repeated dose oral toxicity study in the SD rat	10 SD rats/sex per group; 1,000, 2,500, 5,000 mg 6′-SL/kg bw per day

CHL: Chinese hamster lung; SD: Sprague–Dawley.

The Panel considers the information on 6′-SL when produced by enzymatic synthesis as supportive for the assessment of the NF.

3.10.4. Human data

No human intervention studies with 6′-SL have been provided by the applicant and no reference to human data was made.

3.11. Allergenicity

The protein content in the NF is low as indicated in the specifications (Table 2).

In addition, the applicant has assessed the allergenic potential of introduced proteins as a result of the genetic modification of the *E. coli* K-12 host (which itself is recognised as non-allergenic) using the

search algorithms provided by the Allergen Online tool (version 17) of the University of Nebraska (FARRP, 2017). No sequence alerts for potential allergenicity were identified.

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

4. Discussion

The NF is mainly constituted of the sodium salt of a HiMO, 6′-SL, and other minor fractions (e.g. sialic acid and 6′-sialyl-lactulose). The NF is obtained by microbial fermentation with a genetically modified strain of *E. coli* K-12 DH1 MDO. The information provided on the manufacturing process, composition and specifications of the NF, including the absence of DNA from the producing microorganisms, does not raise safety concerns.

The applicant intends to add the NF to a variety of foods, including IF and follow-on formula, foods for infants and young children, foods for special medical purposes and food supplements. The target population is the general population.

The Panel notes that under acidic conditions, the NF will be hydrolysed to lactose and sialic acid and it will not meet the specifications. The same would apply for 6-sialyl-lactulose formed under alkaline conditions.

The Panel considers that there are no concerns regarding genotoxicity of the NF. In the 90-day oral toxicity study conducted in neonatal rats at the doses of 1,000, 3,000 or 5,000 mg/kg bw per day of the NF a testicular finding (monolateral tubular atrophy) has been observed at the high dose of 5,000 mg/kg bw per day. The Panel agrees with the authors' conclusion identifying the dose of 3,000 mg/kg bw as the NOAEL. When comparing the NOAEL from the 90-day toxicity study (3,000 mg/kg bw) with the highest estimated exposure per population category (at 95th percentile; Table 6), the margins of exposure are relatively low, with range from 16 to 300.

Considering that 6′-SL is a naturally occurring oligosaccharide present in human milk, the history of human exposure to 6′-SL concerns breast-fed infants. 6′-SL is one of the most abundant acidic HMO and one of the most represented HMOs along with 2′-FL, LNT, lacto-*N*-fucopentaose I, disiallylacto-*N*-tetraose and LNnT in human milk. The Panel also notes that other HiMOs (2′-FL, DFL and LNT) produced by fermentation with the same genetically modified *E. coli* strain have been recently assessed (EFSA NDA Panel, 2019a,b).

The Panel notes that the anticipated daily intake of 6′-SL in the NF from the consumption of IF only, in infants up to 16 weeks of age, does not exceed the highest intake level of 6′-SL in breastfed infants on a body weight basis. The anticipated daily intake of the NF for the proposed uses at their respective maximum use levels in the other population categories is not exceeding the highest intake level of 6′-SL in breastfed infants on a body weight basis. Thus, since the intake in breastfed infants on a body weight basis is expected to be safe also for other population groups, the Panel considers that the intake of the NF for the proposed uses at their respective maximum use levels can be considered safe. The maximum daily intake of 6′-SL as a food supplement at the proposed maximum levels (i.e. from 300 mg to 1 g/day) for the respective population categories also does not exceed the highest intake level of 6′-SL in breastfed infants per kg bw. Food supplements are not intended to be used if other foods with added 6′-SL (as well as breast milk for infants and young children) are consumed on the same day.

In terms of foods for special medical purposes, the applicant did not propose maximum use levels and the Panel considers that the maximum use levels of the NF should not lead to higher daily intakes than those estimated on the basis of the maximum levels specified for the proposed food uses or the maximum daily intake proposed for food supplements.

It is finally noted that, as with other oligosaccharides, which are natural components of human milk, the safety assessment is mainly based on the comparison between the natural intake in breastfed infants and the estimated intake as NF. The same considerations apply for lactose and other mono- and oligosaccharides (e.g. sialic acid) that are present as a very small fraction in the NF and considered of no safety concern. 6′-sialyl-lactulose that is not a natural milk component is characterised by very low concentrations not having any laxative effect.

5. Conclusions

The Panel concludes that the NF, composed of 6′-SL sodium salt and other structurally related mono- and oligosaccharides, is safe under the proposed conditions of use, including the use as a food supplement. The target population is the general population.

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

The Panel could not have reached the conclusions on the safety of the NF under the proposed conditions of use without the following data claimed as proprietary by the applicant:

- annexes to the dossier which relate to the identity, the production process, composition and specifications of the NF (see annexes indicated in Section 2.1).
- bacterial reverse mutation test (Unpublished study report, 2018a), *in vitro* micronucleus test (Unpublished study report, 2018b) and 90-day oral toxicity study with the NF (Unpublished study report, 2018d) including the summary table of the statistically significant observations in the 90-day study (Appendix B.3 to the dossier).

Steps taken by EFSA

1) Letter from the European Commission to the European Food Safety Authority with the request for a scientific opinion on the safety of 6′-SL sodium salt as a novel food. Ref. Ares (2019)3216553, dated 16/05/2019.
2) On 16/05/2019, a valid application from the European Commission on 6′-SL as NF, which was submitted by Glycom A/S, was made available to EFSA by the European Commission through Commission e-submission portal (NF 2019/0881) and the scientific evaluation procedure started.
3) On 12/09/2019 and on 16/12/2019, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4) On 02/12/2019 and 20/01/2020, additional information was provided by the applicant and the scientific evaluation was restarted.
5) During its meeting on 23/03/2020, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of 6′-SL sodium salt as a NF pursuant to Regulation (EU) 2015/2283.

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Abbreviations

2′-FL	2′-Fucosyllactose
3′-SL	3′-Sialyllactose
6′-SL	6′-Sialyllactose
ACVA	4,4′-azobis (cyanovaleric acid)
ADME	absorption, distribution, metabolism and excretion
bw	body weight
CAD	charged aerosol detection
CFU	colony forming units
Da	Daltons
DFL	difucosyllactose
DSMZ	German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen)
EMEA	European Medicines Agency
EU	endotoxin units
Eur.Ph.	European pharmacopeia
FAIM	Food Additive Intake Model
FDA	US Food and Drug Administration
Gal	Galactose
Glc	glucose
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis Critical Control Points
HiMS	human identical milk saccharides
HiMO	human identical milk oligosaccharide
HMO	human milk oligosaccharide
HPAEC	high performance anion exchange chromatography
HPLC/CAD	high performance liquid chromatography/charged aerosol detection
HMBC	heteronuclear multiple bond correlation
HSQC	heteronuclear single quantum coherence
IC	Ion chromatography
IF	infant formula
ISO	International Organization for Standardization
K-F	Karl Fischer
LNT	lacto-*N*-tetraose
LNnT	lacto-*N*-neotetraose
LOR	limit of reporting
MS	mass spectrometry
NANA	N acetyl neuraminic acid
NDA	The EFSA Panel on Nutrition, Novel Foods and Food Allergens
NDNS	National Diet and Nutrition Survey
Neu5Ac	N-acetylneuraminic acid
NF	novel food
NMR	nuclear magnetic resonance spectroscopy
NOAEL	no observed adverse effect level
NOESY	Nuclear Overhauser Effect Spectroscopy
NMR	nuclear magnetic resonance spectroscopy

OECD	Organisation for Economic Co-operation and Development
PAD	pulsed amperometric detection
PCR	Polymerase chain reaction
QPS	qualified presumption of safety
RH	relative humidity
RT	retention time
UHT	Ultra-high temperature
UV	ultraviolet