Glycom A/S

Submission on Application A1265:

2'-FL/DFL, LNT, 6'-SL sodium salt and 3'-SL sodium salt as nutritive substances in infant formula products

Submission Date:

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Glycom A/S is a wholly owned indirect affiliate of DSM Nutritional Products Ltd, a company with a registered address at [redacted].

Contact Details

Preamble

Glycom A/S, the Applicant, is appreciative for the opportunity to comment on Application A1265 to amend the Australia New Zealand Food Standards Code (the Code) to permit the voluntary addition of the following four human-identical milk oligosaccharides (HiMOs) produced by microbial fermentation for use as nutritive substances in infant formula products (IFP):

- 2′-Fucosyllactose/difucosyllactose (2′-FL/DFL);
- Lacto-*N*-tetraose (LNT);
- 6′-Sialyllactose (6′-SL) sodium salt; and
- 3′-Sialyllactose (3′-SL) sodium salt.

Glycom A/S, a wholly owned indirect affiliate of DSM Nutritional Products Ltd., is a Danish food ingredient manufacturer who specialises in the development, synthesis and commercialisation of HiMO substances.

We thank FSANZ for their consideration of this Submission on Application A1265.

Expression of Maximum Permitted Use Levels

As requested in the Application, it is recommended that maximum permitted use levels are expressed on a HiMO basis rather than on an ingredient basis. There are several reasons supporting the expression of use levels on a HiMO basis:

- The purity of the active HiMO(s) varies across individual batches, HiMO products, and manufacturers. While the specifications ensure a minimum concentration of the active HiMO(s) in the final ingredient, there can still exist variability in the range above the minimum concentration. Thus, maximum permissible use levels expressed on an ingredient basis can result in different maximum amounts of the active HiMO(s) added to IFP.

For example, the minimum specified sum of 2′-FL and DFL for the 2′-FL/DFL mixture in the draft variation to the Code is not less than 85%. If maximum permitted amounts are expressed on an ingredient basis, 2'-FL/DFL with an assay value of 85% for the sum of 2′-FL and DFL added to IFP at the maximum permitted amount (96 mg/100 kJ) would result in a lower amount of 2′-FL and DFL in the final IFP (81.6 mg/100 kJ) compared to 2′-FL/DFL with a higher assay value (up to 96 mg/100 kJ).

- Mean estimated dietary intakes of 2′-FL, DFL, LNT, 6′-SL and 3′-SL from mature human milk, used to support the safety of the proposed conditions of use of 2′-FL/DFL, LNT, 3′-SL sodium salt and 6′-SL sodium salt as nutritive substances in IFP, are based on concentrations (g/L) of the HMOs analytically measured from mature breast milk samples. As such, the comparison of the estimated dietary intakes of 2′-FL, DFL, LNT, 6′-SL and 3′-SL from infant formula at maximum proposed use levels from the Application to those from mature human milk, assumes 100% purity of the active HiMO(s) in the final HiMO ingredient.

- In infant clinical trials evaluating the safety and benefit of the addition of HiMOs to infant formula, doses are expressed on a pure HiMO basis, analytically measured in the final test formula. This also means that 3′-SL and 6′-SL dosages are expressed on a free acid basis (*i.e.*, without the counter ion).

- Release limits and declarations of HiMOs from IFP are based on the pure nutrient level analytically verified in the final IFP, and are expressed on a free acid basis without the counter ion for 3′-SL and 6′-SL.

- Other authorities consider maximum permitted use levels of HiMOs to be expressed on a HiMO basis, including the U.S. (confirmed *via* personal communication with the U.S. FDA) and the EU [*e.g.*, see EFSA Scientific Opinions on the safety of 3′-SL sodium salt[1] and 6′-SL sodium salt[2] as novel foods and the 3′-SL sodium salt and 6′-SL sodium salt listings in Commission Implementing Regulation (EU) 2015/2283[3]].

[1] Safety of 3′-Sialyllactose (3′-SL) sodium salt as a novel food pursuant to Regulation (EU) 2015/2283. Available from: https://www.efsa.europa.eu/en/efsajournal/pub/6098.
[2] Safety of 6′-Sialyllactose (6′-SL) sodium salt as a novel food pursuant to Regulation (EU) 2015/2283. Available from: https://www.efsa.europa.eu/en/efsajournal/pub/6097.
[3] Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001. Current consolidated version: 27/03/2021. Available from: https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32015R2283.

Sialyllactose Abbreviations

The abbreviations "6'-SL" and "3'-SL" in the Call for Submissions and draft variation to the Code include the sodium salt when addressing the HiMO ingredient. However, the same abbreviations are used when referring to 3'-SL and 6'-SL quantified in human milk, which are usually expressed on a free acid basis.

All acids (including 6'-SL and 3'-SL) occur in aqueous solution in an equilibrium of the free acid and – depending on the pH - the deprotonated acid, which requires a counter-ion. In biological liquids (such as milk) the nature of the counter ion depends on the osmolarity of the electrolytes present in the liquid (*e.g.*, sodium, potassium, magnesium, calcium, and phosphorus). Therefore, and for simplification, acidic substances in aqueous solution are typically referred to by the name of the free acid (*i.e.,* 6'-SL or 3'-SL) - and not as the different co-existing salt forms.

However, when isolating an acidic ingredient as a dry powder, the acid is usually converted into a specific salt form (*i.e.*, 6'-SL sodium salt; 3'-SL sodium salt) since this step increases the stability and homogeneity of the powder.

For the above reasons, it is recommended that the "6'-SL" and "3'-SL" abbreviations do not include the sodium salt, as follows:

- 6'-sialylactose (6'-SL) sodium salt
- 3'-sialylactose (3'-SL) sodium salt

LNnT Listing as Individual Permission

Under Application A1251, FSANZ did not identify any public health and safety concerns on the combined use of 2'-FL with the other non-digestible oligosaccharides galacto-oligosaccharides (GOS) and/or inulin-type fructans (ITF) permitted for use in IFP. In the current risk assessment for Application A1265, FSANZ has similarly concluded that there are no public health and safety concerns with the combined used of LNnT with ITF and/or GOS in IFP at current maximum permitted use amounts. As such, in the draft variation of the Code, FSANZ has proposed the removal of the current prohibition in Standard 2.9.1 on the addition to IFP of GOS and/or ITF in combination with LNnT. Furthermore, FSANZ are not proposing to prohibit the use of GOS and/or ITF in combination with 2'-FL/DFL, LNT, 6'-SL sodium salt and/or 3'-SL sodium salt.

We agree with this amendment, and would further propose that the Code is amended to permit the individual use of LNnT under the same principle of lack of public health and safety concerns on the use of LNnT alone or in combination with HiMOs other than 2'-FL.

LNnT is already permitted for use as a nutritive substance in IFP but currently only in combination with 2′-FL according to section S29—5 of the Code (see below).

S29—5 Infant formula products—substances permitted as nutritive substances

For section 2.9.1—5, the table is set out below.

Infant formula products—substances permitted for use as nutritive substances

Column 1	*Column 2*	*Column 3*	*Column 4*
Substance	*Permitted forms*	*Minimum amount per 100 kJ*	*Maximum amount per 100 kJ*
2'- fucosyllactose permitted for use by Standard 1.5.2	2'- fucosyllactose		96 mg
A combination of: 2'-fucosyllactose permitted for use by Standard 1.5.2; and lacto-N-neotetraose permitted for use by Standard 1.5.2	2'- fucosyllactose and lacto-N-neotetraose		96 mg which contains not more than 24 mg of lacto-N-neotetraose

The maximum amount of LNnT permitted in combination with 2′-FL is 24 mg/100kJ (equivalent to ~ 0.6 to 0.8 g/L[4]), and is within the range of mean concentrations of LNnT reported in mature human milk (see Soyyılmaz *et al.*, 2021). The combined maximum amount of 2'-FL or 2'-FL/DFL, LNT, **LNnT**, 6'-SL and 3'-SL would total **0.18 g/100 kJ** added to IFP and thus remains well below the lower limit of the average total oligosaccharide concentration reported in mature human milk (10 to 15 g/L – Zhang *et al.*, 2021; calculated by FSANZ to range from 0.34 to 0.51 g/100 kJ). Including the maximum amounts permitted for ITF and GOS would equal **0.58 g/100 kJ** of total added oligosaccharides, which is still only a fraction of the total range of carbohydrate content (~ 5 %).

Table 1 Total Added Oligosaccharides to IFP considering the Individual Use of LNnT (Prepared from Table 1 of the FSANZ A1265 CFS Report)

Oligosaccharide	Maximum amount (mg/100 kJ)	Amount expressed as %Energy
2′-FL or 2′-FL/DFL	96	0.8
LNT	32	0.3
LNnT	24	0.2
6′-SL	16	0.1
3′-SL	8	0.1
Total HiMO	**176**	**1.5**
ITF	110	0.9
GOS	290	2.3
Total oligosaccharide	**576**	**4.7**

2′-FL = 2′-fucosyllactose; 3′-SL = 3′-sialyllactose; 6′-SL = 6′-sialyllactose; CFS = Call for Submissions; DFL = difucosyllactose; GOS = galacto-oligosaccharides; HiMO = human-identical milk oligosaccharides; IFP = infant formula products; ITF = inulin-type fructans; LNnT = lacto-N-neotetraose; LNT = lacto-N-tetraose.

[4] Based on energy requirements for infant formula and follow-on formula of 2,500 to 3,150 kJ/L as per Standard 2.9.1—9 of the Code.

In the draft variation to the Code, 2'-FL/DFL, LNT, 6'-SL and 3'-SL are listed as individual permissions in section S29—5, as minimum effective regulation. FSANZ indicate that this approach allows for more efficient assessments of future applications for HiMO-type ingredients and supports international consistency.

LNnT is a separate HiMO ingredient (*i.e.*, it is not sold as a mixture with 2′-FL, unlike DFL). It is already permitted for use as a single ingredient in IFP in countries where permissions exist. Recently in the EU, the obligatory use of LNnT in combination with 2′-FL at a 1:2 ratio has been removed according to Commission Implementing Regulation (EU) 2023/950[5] and Commission Implementing Regulation (EU) 2023/961[6], amending the Union list of authorised novel foods.

Therefore, listing LNnT as an individual permission in the Code would harmonise HiMO permissions in the Code and further support international consistency.

Specifications

- Some parameters were specified as being expressed on a water-free basis in the Application but are not specified as such in the draft variation to the Code (Schedule 3—Identity and purity: S3—47 to S3—50). As the water content varies from batch-to-batch, albeit within the specification limit for water (not more than 6.0 or 8.0% depending on the HiMO product), it is strongly recommended that assay values for the active HiMO(s) and sum of human identical milk saccharides are expressed on a water-free basis.

- The residual protein analysis method (Bradford assay) is not specified in the draft variation to the Code (S3—47 to S3—50). It is recommended that the assay is indicated in the specifications, as residual protein analysis methods based on nitrogen content (*e.g.,* Kjeldahl method) are not appropriate considering some HiMOs contain nitrogen (*e.g.*, LNT, 6′-SL, 3′-SL).

- As previously stated, it is recommended that the "6′-SL" and "3-SL abbreviations do not include the sodium salt. Furthermore, this approach results in the consistent use of the abbreviation across all specification parameters (see 'sum of human identical milk saccharides' parameter).

The proposed amendments are summarised in Table 2 below.

[5] Commission Implementing Regulation (EU) 2023/950 of 12 May 2023 amending Implementing Regulation (EU) 2017/2470 as regards the conditions of use of the novel food 2′-Fucosyllactose. Available from: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32023R0950.
[6] Commission Implementing Regulation (EU) 2023/961 of 12 May 2023 amending Implementing Regulation (EU) 2017/2470 as regards the conditions of use of the novel food Lacto-N-neotetraose. Available from: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32023R0961.

Table 2	Proposed Amendments* to the Draft Variation to the Australia New Zealand Food Standards Code (Schedule 3—Identity and purity)	
S3—47	Specification for a combination of 2′-fucosyllactose and difucosyllactose sourced from *Escherichia coli* K-12	(f) 2′-FL––not less than 75.0% (water-free)
		(g) DFL––not less than 5.0% (water-free)
		(h) sum of 2′-FL and DFL––not less than 85.0% (water-free)
		(i) sum of human identical milk saccharides: 2′-FL, DFL, D-lactose, L-fucose, 3-fucosyllactose ~~FL~~––Not less than 92.0% (water-free)
		(p) residual protein (Bradford assay)––not more than 0.01%
S3—48	Specification for lacto-*N*-tetraose sourced from *Escherichia coli* K-12	For lacto-N-tetraose (LNT) sourced from *Escherichia coli* K-12 containing the gene for beta-1,3-N-acetylglucosaminyltransferase from *Neisseria meningitides* and the gene for beta-1~~,~~3-galactosyltransferase from *Helicobacter pylori*, the specifications are the following:
		(f) LNT––not less than 70.0% (water-free)
		(g) sum of human identical milk saccharides: LNT, D-lactose, lacto-N-triose II–– not less than 90.0% (water-free)
		(j) para-lacto-N-hexaose-2––not more than 3.5%
		(n) residual protein (Bradford assay)––not more than 0.01%
S3—49	Specification for 6′-sialyllactose sodium salt sourced from *Escherichia coli* K-12	(f) 6′-SL sodium salt––not less than 90.0% (water-free)
		(g) sum of human identical milk saccharides: 6′-SL sodium salt, D-lactose, sialic acid––not less than 94.0% (water-free)
		(o) residual protein (Bradford assay)––not more than 0.01%
S3—50	Specification for 3′-sialyllactose sodium salt sourced from *Escherichia coli* K-12	(f) 3′-SL sodium salt––not less than 88.0% (water-free)
		(g) sum of human identical milk saccharides: 3′-SL sodium salt, D-lactose, sialic acid––not less than 90.0% (water-free)
		(o) residual protein (Bradford assay)––not more than 0.01%
(*) Additions are indicated in green text and deletions are indicated in ~~red strikethrough text~~.		

Corrections

Please see in Table 3 below a summary of corrections to the Call for Submissions reports (Call for submissions and Supporting document 1 – Risk assessment).

Table 3 Summary of Corrections* to the Call for Submissions Reports		
Section	**Revision**	**Comment**
CFS Report: 1.4 International Standards	2'-FL/DFL, LNT, 6'-SL and 3'-SL are also authorised for use as novel food ingredients in the UK.	In the UK, 2'-FL/DFL and LNT are authorised for use as novel food ingredients for foods including infant formula and follow on formula under retained EU law, and 6'-SL and 3'-SL have been authorised for use as novel food ingredients for foods including infant formula and follow on formula under: • The Novel Foods (Authorisations) and Smoke Flavourings (Modification of Authorisations) (England) Scotland, Wales) Regulations 2022; • The Novel Foods (Authorisations) and Smoke Flavourings (Modification of Authorisations) (Scotland) Regulations 2022; and • The Novel Foods (Authorisations) and Smoke Flavourings (Modification of Authorisations) (Wales) Regulations 2022. See Part 3.3.3, Section C.3.2 of the Application.

Table 3 Summary of Corrections* to the Call for Submissions Reports		
Section	**Revision**	**Comment**
	"FSANZ notes that another manufacturer of HiMO substances has recently gained EU approval for LNT, 3-fucosyllactose (3~~'~~-FL), 6'-SL and 3'-SL to be used in infant formula products and other foods."	3-FL is spelt without a prime symbol to indicate that the fucose unit is attached to the glucose unit of lactose, according to HMO nomenclature[1].
SD 1: Executive summary	"Two human clinical studies with infants fed formula containing 5.75 g/L of an alternative mixture of five HiMOs, including 2′-FL, LNT, 6′-SL and 3′-SL, also found it was well tolerated and did not affect growth."	Manufactured HiMOs were evaluated in the clinical studies.
SD 1: 3.2.3 Toxicological studies with LNT	"The same test item was used in all toxicity and genotoxicity studies of LNT (Batch No. CPN4215 1000416 FD; 77.0% w/w LNT, water-free)."	The LNT assay value is reported on a water-free basis.
SD 1: 3.2.3 Toxicological studies with LNT; 90-day toxicity study in neonatal rats	"An additional reference control group (10/sex/group) was administered 4000 ~~5000~~ mg/kg bw/day oligofructose."	The reference control group received fructooligosaccharides powder at a dose of 4000 mg/kg bw/day, to allow for direct comparison against the high-dose LNT group (see Phipps *et al*. 2018).
	"Six animals died or were killed for welfare reasons during the study: one male and one female from the vehicle control group, one reference control female ~~male~~, one male and one female given 1000 mg/kg bw/day LNT and one female given 4000 mg/kg bw/day LNT."	One female from the reference control group died during the study (see Phipps *et al*. 2018).
SD 1: 3.2.4 Toxicological	"The same test item was used in all toxicity and genotoxicity studies of 6′-SL	The 6′-SL batch used for the safety studies was CPN5315 1000317 FD, and the 6′-SL assay

Table 3 Summary of Corrections* to the Call for Submissions Reports		
Section	**Revision**	**Comment**
studies with 6′-SL	produced by the Applicant (Batch No. ~~CPN5615~~ CPN5315 1000317 FD; 96.8% w/w 6′-SL, water-free)."	value is reported on a water-free basis.
SD 1: 3.2.4 Toxicological studies with 6′-SL; 90-day oral gavage toxicity study in neonatal rats	"Flaxmer 2018b ~~The study author~~ considered it was not possible to conclusively rule out a test item-related effect, as the incidence was outside the historical control range for studies of this type performed at the laboratory. On this basis they ~~the study author~~ concluded the mid-dose, 3000 mg/kg bw/day, was the NOAEL. FSANZ considers the available evidence indicates these changes were highly unlikely to be treatment-related, however, and concluded the NOAEL was 5000 mg/kg bw/day 6′-SL, the highest dose tested."	Phipps *et al.* 2019 concluded the high-dose (5000 mg/kg bw/day) was the NOAEL, while Flaxmer 2018 concluded the mid-dose (3000 mg/kg bw/day) was the NOAEL.
SD 1: 3.2.5 Toxicological studies with 3′-SL	"The same test item was used in all toxicity and genotoxicity studies of 3′-SL produced by the Applicant (Batch No. CPN5115 1000516 FD; 90.3 % w/w 3′-SL, water-free)."	The 3′-SL assay value is reported on a water-free basis.
SD 1: 3.2.9 Summary of the toxicology assessment	"In a human clinical study, consumption of infant formula containing 1.5 or 2.5 g/L of a mixture of 2′-FL/DFL, LNT, 6′-SL and 3′-SL, followed by follow-up formula and growing-up milk containing 0.5 g/L or 0.4 g/L of the HiMO blend, respectively, was safe and well tolerated."	Manufactured HiMOs (not HMOs isolated from human milk) were evaluated in the clinical studies.

Table 3 Summary of Corrections* to the Call for Submissions Reports		
Section	**Revision**	**Comment**
	"Two human clinical studies with infants fed formula containing 5.75 g/L of an alternative mixture of 5 HiMOs, including 2′-FL, LNT, 6′-SL and 3′-SL, also found the formula was safe and well tolerated."	
SD 1: 3.4.1.3 Concentrations of 2′-FL, DFL, LNT, 6′-SL and 3′-SL in infant formula products; Table 8	Mean and high 2′-FL and DFL concentrations used for FSANZ dietary intake assessment (mg/100 kJ) are inverted **2′-FL** Mean = 84.5 High = 91.2 **DFL** Mean = 11.5 High = 24.0	Typo.
SD 1: 3.4.2 Dietary intake assessment methodology	"~~For assessment of milk oligosaccharides in cows' and goats' milk, the energy content values of 281 kJ/100 g for Milk, cow, fluid, regular fat (~3.5%) and 207 kJ/100 g for Milk, goat, fluid, regular fat were used, respectively (FSANZ 2016).~~"	Suggest removal as "(…) a comprehensive dietary intake assessment of milk oligosaccharides from cows' or goats' milk was therefore not undertaken for this Application." (see Section 3.4.1.5).
SD 1: 3.4.2.1 Assumptions and limitations of the dietary intake assessment	"~~1 litre of cows' or goats' milk equals 1,030 grams~~"	Suggest removal as "(…) a comprehensive dietary intake assessment of milk oligosaccharides from cows' or goats' milk was therefore not undertaken for this Application." (see Section 3.4.1.5).
SD 1: 3.4.3.1 FSANZ estimated dietary intakes from infant/follow-	P90 (mean) estimated dietary intake (g/kg bw/day) of 2′-FL should be 0.58 ~~0.058~~.	Typo.

Table 3 Summary of Corrections* to the Call for Submissions Reports		
Section	**Revision**	**Comment**
on formula and human milk; Table 12		
SD 1: 3.4.3.1 FSANZ estimated dietary intakes from infant/follow-on formula and human milk; Table 13	Mean (mean) estimated dietary intake (g/kg bw/day) for LNT should be 0.091 ~~0.085~~.	Typo.
SD 1: 3.4.3.1 FSANZ estimated dietary intakes from infant/follow-on formula and human milk; Tables 14 & 15	"Assuming P50 body weight of 8.9 kg (World Health Organization 2006), recommended energy intake of 330 kJ/kg bw/day (United Nations University and World Health Organization 2004) and 50 ~~100~~% of dietary energy is obtained from follow-on formula."	As infants aged 9 months were assumed to consume follow-on formula or human milk in amounts that meet 50% of their energy requirements. Typo. Otherwise, values in the table are correct.
SD 1: 4.4 Effect of individual HiMOs on infant growth	"No human intervention studies for 6'-SL, 3'-SL, LNT and DFL as single HiMOs were provided by the Applicant. A search of the literature identified only studies where test formulas contained various mixtures of the relevant HiMOs (Parschat et al. 2021; Bosheva et al. 2022; Lasekan et al. 2022) and we were unable to identify additional published clinical studies testing the effects of 6'-SL, 3'-SL, LNT and DFL as individual HiMOs on infant growth." "Therefore, based on the	No published clinical studies have tested the individual effects of LNT on infant growth. LNT has been evaluated in combination with other HiMOs in human intervention studies (Parschat *et al.* 2021; Bosheva *et al.* 2022; Lasekan *et al.* 2022), similar to 6'-SL, 3'-SL and DFL.

Table 3 Summary of Corrections* to the Call for Submissions Reports		
Section	**Revision**	**Comment**
	best available evidence, addition of individual HiMOs (3′-SL, 6′-SL, LNT and DFL), or different mixture of these to that presented in section 4.3, would be unlikely to adversely affect infant growth as long as the HiMOs are added at levels that are normally found in human milk."	
SD 1: Table A-2 Summary of human intervention trials	"Lasekan (~~2021~~ 2022) United States (multisite)"	Typo.
	Cohen (2022, unpublished) "Randomised to 3 formula groups: 693 ~~686~~" "Growth: Co-Primary outcome: weight gain per day from V1 to V6 (4 mo period)." "Study duration to 15 ~~12~~ months."	The full duration of the clinical trial is 15 months (see NCT 03722550). Safety-related data are available up to 12 months of age (Cohen 2022, unpublished). Benefit-related data are available up to 6 months of age (Bosheva *et al.*, 2022). The clinical trial had two, co-primary, outcomes (see NCT 03722550): 1. To compare the growth of infants between the groups (from Study Day 1 to 4 months of age) 2. To compare recurrent incidences of illness of infants between the groups (from Study Day 1 to 15 months of age)

(*) Corrections are indicated in green text (additions) or in ~~red strikethrough text~~ (deletions).

[1] McNaught, A. (1996). Nomenclature of carbohydrates (IUPAC Recommendations 1996). Pure and Applied Chemistry, 68(10), 1919-2008. https://doi.org/10.1351/pac199668101919.

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