EXECUTIVE SUMMARY

Glycom A/S[1] (referred to as "Glycom" hereafter) is seeking to amend the Australia New Zealand Food Standards Code (the Code) for the use of four human-identical milk oligosaccharide (HiMO) ingredients produced by microbial fermentation, alone or in combinations, as nutritive substances in infant formula products. These include a mixture of 2'-fucosyllactose (2'-FL) and difucosyllactose (DFL) ("2'-FL/DFL"), lacto-*N*-tetraose (LNT), 6'-sialyllactose (6'-SL) sodium salt, and 3'-sialyllactose (3'-SL) sodium salt, capturing the 3 major types of human milk oligosaccharides (HMOs) that occur naturally in human milk. The addition of 2'-FL/DFL, LNT, 6'-SL sodium salt, and 3'-sialyllactose (3'-SL) sodium salt to infant formula products is consistent with efforts to produce products that better match the nutrient composition of human breast milk, as set forth by principles in the Australia and New Zealand Food Regulation Ministerial Council's Policy Guideline on the Regulation of Infant Formula Products and the Codex Standard for Infant Formula and Formulas for Special Medical Purposes Intended for Infants (Codex Stan 72). As such, the maximum intended use levels of 2'-FL/DFL, LNT, 6'-SL sodium salt, and 3'-SL sodium salt in infant formula products, specified on a HiMO basis in Table 1, are proposed on the basis of providing similar levels of 2'-FL, DFL, LNT, 6'-SL, and 3'-SL as those occurring on average in human milk.

Table 1 Maximum Proposed Use Levels of 2'-FL/DFL, LNT, 6'-SL Sodium Salt, and 3'-SL Sodium Salt (on a Specified HiMO Basis) in Infant Formula Products

Ingredient	Specified HiMO(s)	Maximum Amount of specified HiMO(s)	
		per 100 KJ	per L
2'-FL/DFL Mixture	2'-FL + DFL	96 mg	2.4
LNT	LNT	32 mg	0.8
6'-SL sodium salt	6'-SL	16 mg	0.4
3'-SL sodium salt	3'-SL	8 mg	0.2

2'-FL/DFL = 2'-fucosyllactose/difucosyllactose mixture; 3'-SL = 3'-sialyllactose; 6'-SL = 6'-sialyllactose; LNT = lacto-*N*-tetraose.

Glycom's manufactured 2'-FL/DFL, LNT, 6'-SL, and 3'-SL are chemically and structurally identical to the same molecules that are naturally present in human breast milk.

The four HiMO ingredients are produced from the same *Escherichia coli* (*E. coli*) K-12-derived platform strain as Glycom's 2'-FL and LNnT already authorised for use in Australia and New Zealand. The production strains vary in the introduction of well-characterised donor genes necessary for the biosynthesis of 2'-FL and DFL, LNT, 6'-SL, and 3'-SL, derived from *de novo* DNA synthesis based on defined DNA sequences obtained from bioinformatic databases. The production organisms will not enter Australia or New Zealand as 2'-FL/DFL, LNT, 6'-SL sodium salt, and 3'-SL sodium salt will be manufactured in Denmark under contained use of the genetically modified microorganism.

Glycom manufactures 2'-FL/DFL, LNT, 6'-SL sodium salt, and 3'-SL sodium salt in compliance with current Good Manufacturing Practice (cGMP) and the principles of Hazard Analysis Critical Control Point (HACCP). The manufacturing process is similar for all four ingredients and can be broadly divided into 2 stages. The first stage (upstream processing) consists of a controlled fermentation process where the production organism with adapted cellular metabolism of 2'-FL/DFL, LNT, 6'-SL sodium salt, or 3'-SL sodium salt is incubated with lactose (the substrate) and a carbon energy source (glucose, glycerol, or sucrose). The second stage (downstream processing) consists of the removal of the production organism and the purification 2'-FL/DFL, LNT, 6'-SL sodium salt, or 3'-SL sodium salt released into the fermentation broth. No solvents are used in the manufacturing process as there is no crystallisation step, resulting in non-crystallised 2'-FL/DFL, LNT, 6'-SL sodium salt, or 3'-SL sodium salt products with a minimum purity ranging between 70.0 and 90.0 w/w %. The remaining fraction primarily consists of lactose (the substrate) and other related and fully characterised carbohydrates produced during the fermentation process, most of which are naturally occurring components of human milk. Food grade specifications established by Glycom include these fermentation by-products and other trace elements, as well as microbiological parameters to ensure

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the purity of the final product. Batch analyses demonstrate that the manufacturing process consistently produces 2'-FL/DFL, LNT, 6'-SL sodium salt, or 3'-SL sodium salt that is compliant with appropriate food grade specifications.

HMOs, including 2'-FL, DFL, LNT, 6'-SL, and 3'-SL, do not undergo any significant digestion in the upper gastrointestinal tract but are instead fermented in the colon by intestinal microbiota or are excreted unchanged in the faeces. A small proportion of ingested HMOs may be absorbed intact and excreted in the urine.

Glycom's 2'-FL/DFL, LNT, 6'-SL sodium salt, and 3'-SL sodium salt have each been tested in a comprehensive series of toxicological studies, including a bacterial reverse mutation assay, an *in vitro* mammalian cell micronucleus test in human lymphocytes, and an adapted sub-chronic (90-day) oral toxicity study in neonatal rats. These studies, which have been conducted in compliance with the Organization for Economic Co-operation and Development (OECD) Principles of Good Laboratory Practice (GLP) and relevant OECD Test Guidelines, as well as toxicological studies conducted on other HiMO preparations, demonstrate that these ingredients do not pose any toxicological concerns.

Three randomised controlled clinical studies have been conducted in healthy term infants receiving formula supplemented with 5 HiMOs (2'-FL, LNT, 6'-SL, and 3'-SL in combination with DFL or 3'-fucosyllactose) at levels naturally occuring in human milk. All studies reported non-inferior growth (the primary outcome) during the first 4 months of age in infants receiving the infant formula supplemented with HiMOs (up to 5.75 g/L) compared to infants receiving the control infant formula without HiMOs. Specifically, one of the infant clinical studies evaluated supplementation of Glycom's 2'-FL/DFL, LNT, 6'-SL sodium salt, and 3'-SL sodium salt in infant formula provided to infants from enrolment (7 to 21 days of age) to 6 months of age (1.5 or 2.5 g/L total HiMOs), and in follow-on formula provided to infants from 6 to 12 months of age (0.5 g/L total HiMOs). Additional growth indices, tolerance outcomes, and safety were similar between all formula-fed groups up to 12 months of age.

Other authoritative bodies with comparable regulatory processes to Australia and New Zealand (European Union/European Food Safety Authority, Israel Ministry of Health, Singapore Food Agency, United Kingdom Food Standards Agency/Food Standards Scotland, Brazilian Health Regulatory Agency) have evaluated the safety of Glycom's 2'-FL/DFL, LNT, 6'-SL sodium salt, and 3'-SL sodium salt produced by microbial fermentation and authorised their use in products intended for infants up to 12 months of age. In the United States (U.S.), Glycom's 2'-FL/DFL, LNT, 6'-SL sodium salt, and 3'-SL sodium salt intended for use in infant formula and follow-on formula have been notified as GRAS to the U.S. FDA, to which the Agency provided "no questions" response letters indicating that the notices provide a sufficient basis for GRAS determination.

Intakes of 2'-FL, DFL, LNT, 6'-SL, and 3'-SL from the proposed conditions of use of 2'-FL/DFL, LNT, 6'-SL sodium salt, and 3'-SL salt (as summarised in Table 1) were estimated on an energy basis in infants 3 and 9 months of age using the same model diets as those used by FSANZ for 2'-FL and LNnT. As maximum proposed use levels of 2'-FL/DFL, LNT, 6'-SL sodium salt, and 3'-SL sodium salt in infant formula products (on a HiMO basis) are similar to mean concentrations of the corresponding HMOs in human milk, dietary intakes of the HiMOs from infant formula are similar to the dietary intakes of these same HMOs from human milk.

A bifidogenic effect of 2'-FL and LNnT and pathogen binding effect of 2'-FL against *Campylobacter jejuni* has previously been recognised during the evaluation of Application A1155. In addition to microbiota modulating effects and protective effects against pathogens, the evidence suggests that 2'-FL/DFL, LNT, 6'-SL, and 3'-SL (individually and in combination) also help maintain intestinal barrier integrity and play a role in immunomodulation. Specifically, in the infant clinical study evaluating supplementation of Glycom's 2'-FL/DFL, LNT, 6'-SL sodium salt, and 3'-SL sodium salt in infant formula, the gut microbiome of infants receiving the test formulas containing the HiMOs more closely resembled the gut microbiome of breastfed infants (data available through to 6 months of age). Compared to infants receiving the control formula without HiMOs, infants receiving the test formula with HiMOs had an increased relative abundance of faecal bifidobacteria (notably, *Bifidobacterium longum ssp. infantis*) and a decreased prevalence and relative abundance of faecal pathogenic bacteria (notably, toxigenic *Clostridioides difficile*). Furthermore, infant formula supplemented with the HiMOs was reported to support the development of the intestinal immune system and a healthy gut barrier, assessed using faecal markers of immune and gut health.

Overall, the available scientific evidence, together with the history of safe consumption of 2'-FL, DFL, LNT, 6'-SL, and 3'-SL from human milk, supports the safe use of Glycom's 2'-FL/DFL, LNT, 6'-SL sodium salt, and 3'-SL sodium salt for their intended use as nutritive substances in infant formula products. These HiMOs, representing the 3 major types of HMOs in human milk, have also been demonstrated to have a functional role in the development of

the infant gut. Thus, their addition to infant formula is intended to provide formula-fed infants more similar health benefits to breastfed infants. It is anticipated that the approval of these HiMOs produced by microbial fermentation as nutritive substances for addition to infant formula products in Australia and New Zealand will benefit consumers and industry alike by allowing for the increased availability of innovative products that more closely mimics the composition of human breast milk.