Application for the Approval of 2'-*O*-Fucosyllactose (2'-FL) and Lacto-*N*-neotetraose (LNnT) Under Standard 1.5.1 (Novel Foods) of the Australia and New Zealand Food Standards Code

EXECUTIVE SUMMARY

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Glycom A/S (referred to as "Glycom" hereafter) is seeking to amend Schedule 25 of the Australia New Zealand Food Standards Code to include 2'-*O*-fucosyllactose (2'-FL) and lacto-*N*-neotetraose (LNnT) as novel foods for use as ingredients in infant formula, follow-on formula, and formulated supplementary foods for young children (specifically milk products). 2'-FL is intended to be added to these products at a maximum use level of 1.2 g/L, as consumed. 2'-FL may be added on its own, or in combination with up to 0.6 g/L of LNnT. 2'-FL is a trisaccharide consisting of L-fucose, D-galactose, and D-glucose, whereas LNnT is a tetrasaccharide consisting of D-galactose, *N*-acetyl-D-glucosamine, D-galactose and D-glucose. Both of these oligosaccharides occur naturally in human breastmilk (collectively termed "human milk oligosaccharides" or HMOs), with 2'-FL representing the most abundant HMO. In pooled breastmilk samples, 2'-FL represents up to 15 to 20% (w/w) of the total HMO biomass.

HMOs, including 2'-FL and LNnT, do not undergo any significant digestion in the upper gastrointestinal tract, and therefore serve as growth substrates for the gut microflora. Glycom's 2'-FL and LNnT ingredients are intended to complement the range of other non-digestible oligosaccharide ingredients such as inulin-type fructans and galacto-oligosaccharides that are already permitted for addition to foods for infants and young children in Australia/New Zealand. The addition of 2'-FL, either alone or in combination with LNnT, to infant formula and follow-on formula is consistent with efforts to produce products that better match the nutrient composition of human 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). Such products have been demonstrated to be safe. Additionally, the functional properties of 2'-FL and LNnT may confer a number of beneficial effects on health.

The 2'-FL and LNnT ingredients manufactured by Glycom (obtained by either chemical synthesis and fermentation processes) have gained approval and notification in the European Union as novel food ingredients for use in a variety of food products including infant formulae, follow-on formulae, processed cereal-based food and baby food for infants and young children, milk-based drinks and similar products intended for young children, dietary foods for special medical purposes and meal replacements as well as a range of other food groups for the general population. They have also successfully achieved Generally Recognized as Safe (GRAS) status for these same food uses in the United States (U.S.), which has been notified to the Food and Drug Administration (FDA) without questions. Just recently, 2'-FL and LNnT have been granted approval for use in infant formula and growing up milks in Singapore, and 2'-FL has been authorised for use in infant formula in Israel. A controlled fermentation process with an *Escherichia coli* K-12-derived strain is used to obtain 2'-FL, which is secreted into the culture medium. A series of purification steps are applied to completely remove the intact production organism and result in a highly purified product (*i.*e., containing not less than 94.0% w/w 2'-FL by assay and not less than 96.0% w/w human-identical milk saccharides). LNnT also is produced by a similar process as 2'-FL, using an *E. coli* K-12-derived strain that has been optimised for the biosynthesis of LNnT. The final highly purified product contains not less than 92.0% w/w LNnT by assay and not less than 95% w/w of the sum of human-identical milk saccharides. Glycom manufactures 2'-FL and LNnT in accordance with Good Manufacturing Practice (GMP) and Hazard Analysis Critical Control Point (HACCP) principles. Of note, the 2'-FL and LNnT ingredients will not be manufactured within Australia/New Zealand; rather, only the finished ingredient or food products containing it will be imported. The 2'-FL and LNnT obtained by fermentation is chemically and structurally identical to 2'-FL and LNnT, respectively, that is present in human breast milk. Batch analyses demonstrate that the manufacturing process consistently produces 2'-FL and LNnT that are compliant with appropriate food grade specifications. The impurity profile of these ingredients has been thoroughly characterized and the finished products are free from potential manufacturing contaminants.

The maximum intended use level of 2'-FL at 1.2 g/L, either alone or in combination with up to 0.6 g/L of LNnT, in formula products is intended to directly reflect the level of 2'-FL and LNnT that occurs in human breast milk. Among pooled milk samples, the average content of 2'-FL has been reported at 3.2 g/L in colostrum (**range: 1.0 to 8.4 g/L**), 2.5 g/L in transitional milk (**range: 2.1 to 2.8 g/L**), 2.2 g/L in mature milk from days 10 to 60 of lactation (**range: 0.70 to 3.9 g/L**), and 1.9 g/L in mature milk from 2 months onward (**range: 0.70 to 3.4 g/L**). The average levels of LNnT in pooled milk are highest in colostrum (0.34 g/L; **range: 0.21 to 0.49 g/L**), followed by transitional milk (0.32 g/L; **range: 0.15 to 0.55 g/L**) and continue to decline slowly in mature milk (0.31 g/L; **range: 0.09 to 1.08 g/L**) and mature milk from a lactation stage later than 2 months (0.28 g/L; **range: 0.04 and 1.08 g/L**). Among Secretor mothers, these ranges are observed to be higher, with levels of 2'-FL of up to 7.8 g/L in mature milk and levels of LNnT of up to 0.36 g/L in mature milk. The intended use of 2'-FL, either alone or in combination with LNnT, in infant formula and follow-on formula products are therefore considered safe on the basis that resultant intake levels is supported by similar levels of intake of 2'-FL and LNnT from human breastmilk.

As detailed in this application, the safety of 2'-FL and LNnT can also be supported by an extensive dataset from both preclinical toxicological studies and clinical studies. In studies that were conducted in healthy full-term infants, the administration of formula containing 2'-FL or LNnT at concentrations of up to 1.2 g/L and 0.6 g/L, respectively, was safe, well-tolerated and supported appropriate growth. Similarly, bolus administration of 2'-FL or LNnT to adults has been demonstrated to be well tolerated at doses of up to 20 g/day. A no-observed-adverse-effect level (NOAEL) of 5,000 mg/kg body weight/day for 2'-FL and for LNnT can be supported by several 90-day oral toxicity studies that have been conducted in rats. Further, in a 3-week feeding study conducted in neonatal piglets, no adverse effects were observed when 2'-FL was administered in a milk replacer at doses providing up to 291.74 mg/kg body weight/day in males and 298.99 mg/kg body weight/day in females for a 20-day period. No evidence of mutagenicity/genotoxicity were observed when 2'-FL or LNnT was evaluated in a battery of *in vitro* assays.

Overall, the available scientific evidence, together with the history of safe consumption of 2'-FL and LNnT from human breastmilk, supports the safe use of 2'-FL and LNnT for their intended applications in infant formula, follow-on formula, and formulated supplementary foods for young children. It is anticipated that approval of 2'-FL and LNnT as a novel food in Australia/New Zealand will benefit consumers and the industry alike by allowing for the increased availability of innovative products that more closely mimics the composition of human breast milk.