Application for the Approval of 2'-Fucosyllactose (2'-FL) Use as Nutritive Substance of the Australia and New Zealand Food Standards Code

EXECUTIVE SUMMARY

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Synaura Biotechnology (Shanghai) Co., Ltd. (referred to as "Synaura" hereafter) is seeking permission to amend Schedule 26 (Food produced using gene technology) of the Australia New Zealand Food Standards (FSANZ) Code (the Food Standards Code) to include Synaura's 2'-Fucosyllactose (2'-FL) substance as an nutritive substance in infant formula (from birth to <12 months of age), follow-on formula (from 6 months to <12 months of age), and infant formula for special dietary uses which apply to Australia only.

In this application, the Synaura substance will be referred to as "Synaura 2'-FL" to distinguish it from naturally occurring 2'-FL. Synaura intends to provide Synaura 2'-FL produced from genetically engineered *Escherichia coli* B strain (*Escherichia coli* EB011065 strain) to manufacturers for use in infant and toddler formulas in Australia and New Zealand. The Synaura 2'-FL substance will be commercially available in powder with identical sugar compositions 2'-FL content. The product will be marketed as "2'-fucosyllactose powder" respectively.

Synaura manufactures their 2'-FL using a genetically engineered *Escherichia coli* B strain (*Escherichia coli* EB011065 strain) as a processing aid. The fermentation process does not use antibiotics nor any other inhibitors, and the manufacturing process does not use organic solvents or other toxic substances. The production process is current Good Manufacturing Practice (cGMP), ISO 22000 and fulfills the requirement for food standards. Synaura 2'-FL is a standardized preparation produced by fermentation using a genetically engineered *Escherichia coli* EB011065 strain, and contains not less than 94% 2'- FL. It is a white powder that primarily consists of 2'-FL and minor quantities of other chemically related sugars or by-products. 2'-FL is a fucosylated, neutral trisaccharide composed of L-fucose, D-galactose, and D-glucose units. The molecular constitution of 2'-FL can be described as consisting of the monosaccharide L-fucose and the disaccharide D-lactose, which are linked by α-(1→2) bond to form the trisaccharide. It is a trisaccharide that occurs only as one specific constitutional isomer. Analyses demonstrate that the Synaura 2'-FL does not contain any recombinant DNA material or proteins. Synaura demonstrated and confirmed the purity of the product and

its manufacturing consistency by meeting specifications with five unique production batches, as demonstrated in the certificates of analysis.

Synaura 2'-FL is 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 to infant formula, follow-on formula, and infant formula for special dietary use 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. Such products have been demonstrated to be safe. Additionally, the functional properties of 2'-FL may confer several beneficial effects on health.

2'-FL is a naturally occurring oligosaccharide found in human milk and is one of the most abundant human milk oligosaccharides (HMOs). The intended benefit of adding Synaura 2'-FL to infant formula, follow-on formula, and infant formula for special dietary use is as a nutrient necessary for the body's nutritional and metabolic processes. Consistent with naturally occurring HMOs, Synaura 2'-FL does not undergo any significant digestion in the upper gastrointestinal tract, and serves as a prebiotic for commensal gut bacteria. These bacteria metabolize prebiotics into short-chain fatty acids, which are used by colonocytes in energy production and as a stimulant for sodium and water absorption. Scientific evidence suggests additional infant health benefits from consumption of 2'-FL and other HMOs which include:

- inhibiting the adhesion of pathogenic bacteria and norovirus virus-like particles;
- beneficial impacts on learning and memory;
- reduction in inflammatory intestinal cells;
- enhancing innate immunity to respiratory syncytial virus;
- positively impacting the maturation of cultured intestinal cells and gut motility; and
- a reduction in respiratory and gastrointestinal illness.

The Synaura 2'-FL manufactured by Synaura have already approved in China that used in milk powder (milk powder for children only), infant formula, formula for older

infants and toddlers, and infant formula for special medical purposes. They have also applied and got approval of Generally Recognized as Safe (GRAS) status for these same food uses in the United States (U.S.). What's more, Synaura also has apply registration 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.

Synaura 2'-Fucosyllactose (Synaura 2'-FL) is intended to be used as a food ingredient in milk and soy-based, infant formula (from birth to <12 months of age), and follow-on formula (from 6 months to <12 months of age) at a maximum level of 2.4 g/L of formula as consumed (ready-to-drink or reconstituted formula prepared from powder), in infant formula for special dietary use at a maximum level of 2.4 g/L of formula as consumed (ready-to-drink or reconstituted formula prepared from powder) which apply to Australia only.

2'-FL is a naturally occurring oligosaccharide in human milk and is synthesized from lactose in the mammary gland. The available information suggests that humans, particularly infants, are exposed to 2'-FL either through the ingestion of human milk, cow's milk, and/or products containing synthetic forms of 2'-FL. This also suggests that at naturally found levels, 2'-FL is unlikely to cause any adverse effects. As detailed in this application, the safety of Synaura 2'-FL can also be supported by an extensive dataset from both preclinical toxicological studies and clinical studies. The 2'-FL ingredients manufactured by Synaura has applied for use in the EU and U.S. in the same food categories (i.e., infant formula, follow-on formula, and "toddler milks") and the same (or even higher) use levels as those proposed in Australia/New Zealand. No safety concerns are associated with the use of the source organisms, *Escherichia coli* EB011065 strain, that are used to produce 2'-FL, respectively. HMOs, including 2'-FL, do not undergo any significant digestion in the upper gastrointestinal tract, and are expected to enter the colon largely intact where they are subject to fermentation by the microbiota present.

The safety of 2'-FL has been extensively evaluated in several preclinical and clinical studies. The pre-clinical toxicological dataset for 2'-FL includes metabolic fate, acute toxicity study of 2'-FL, mutagenicity and genotoxicity studies of 2'-FL, teratogenicity study of 2'-FL, multiple studies that include genotoxicity, sub chronic toxicity, neonatal piglet tolerance supports the safety of 2'-FL. The available metabolism related information suggests that 2'-FL is unlikely to cause any adverse effects at the proposed use levels. In an acute toxicity study stated that under the test conditions of the laboratory, the maximum tolerated dose (MTD) in the acute oral toxicity test of 2'-FL (the test article) in female and male ICR mice was found to be greater than 10.00 g/kg bw, and its LD_{50} was greater than 10.00 g/kg bw, indicating that the test article was actually non-toxic. In the mutagenicity and genotoxicity potentials of 2'-FL studies suggest that as evaluated by bacterial reverse mutation test, mammalian erythrocyte micronucleus test, and chromosome aberration test in mouse spermatogonia 2'-FL is unlikely to cause mutagenic or genotoxic effects. In a 90-day repeat dose study, according to the comprehensive analysis, no adverse effects of the test article (2'-FL) in SD rats were found under the test conditions, and the no-observed-adverse-effect level (NOAEL) was 8.00 g/kg bw/day (CAIQTEST, 2022d). In a teratogenicity study, concluded that no potential teratogenic toxicity of the test article was observed in SD rats, under the study conditions. The NOAEL of 2'-FL for teratogenic effects was established as 8.00 g/kg bw/day (CAIQTEST, 2022d). And in other multiple studies suggest that 2'-FL alone or in combination with other HMOs, is not mutagenic, clastogenic or aneugenic, has a NOAEL of at least 5 g/kg bw/day in rats, and is well-tolerated at levels up to 1.6 g/kg bw in neonatal piglets.

In clinical studies include human studies in infants, children, and adults. In the available studies, health, term infants or toddlers were fed formula containing 0.25 to 3 g 2'-FL/L for 6 weeks to 6 months and the findings from these studies suggest that 2'-FL is safe and well-tolerated. In studies in adults, the daily supplementation of up to 20 g 2'-FL is found to be safe and well-tolerated in adults. In addition to the above mentioned studies, support for the safe use of 2'-FL in food for infants and adults is based on results of numerous clinical studies that evaluated the safety and tolerance of HMOs, including 2'-FL, as well as other non-digestible carbohydrates in infants, adults, sensitive populations and oral electrolytes solutions. The available information suggests

that HMOs are well tolerated in infants at levels up to 1 g/day, and in adults at levels up to 20 g/day.

In the safety study of the production microorganism, the available information shows that *E. coli* are commensal residents of the gut microflora of humans and several other animal species. In several comprehensive studies, the safety of *E. coli* BL21 (DE3) has been demonstrated. The *E. coli* BL21 (DE3) does not carry the well-recognized pathogenic components required by *E. coli* strains that cause the majority of enteric infections. Hence, *E. coli* BL21 (DE3) is considered to be nonpathogenic (non-virulent) and unlikely to survive in host tissues or to cause disease (Chart et al., 2000). It should be noted that *E. coli* EB011065 was engineered with genes with known functions, which do not confer toxigenicity, virulence, or DNA, using site-specific homologous recombination. Thus, it can be concluded that *E. coli* EB011065 is non-toxigenic, not capable of DNA transfer to other organisms, and has the same virulence profile (non-pathogenic) as *E. coli* BL21 (DE3). Based on the comprehensive characterization of this strain and its widespread use in protein production, the use of *E. coli* BL21 (DE3) as the host strain and *E. coli* EB011065 as the production strain are not expected to result in any safety concerns.

Overall, the available scientific evidence, together with the history of safe consumption of 2'-FL from human breastmilk, supports the safe use of 2'-FL for their intended applications in infant formula, follow-on formula, and infant formula for special dietary uses. It is anticipated that approval of 2'-FL as a nutritive substance in infant formula products applies to Australia only, and as nutritive substances 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.