Application to amend the Australia New Zealand Food Standards Code Schedule 26 Schedule 26 – Food produced using gene technology to permit "2'-fucosyllactose from the source organism, *Corynebacterium glutamicum* containing the gene for alpha-1,2-fucosyltransferase from *Corynebacterium urelyticum*"

Executive Summary

The purpose is to permit 2'-fucosyllactose (2'-FL) produced from a new genetically modified source organism to be used as a nutritive substance in infant formula products.
To amend Schedule 26 to add "*2'-fucosyllactose from the source organism Corynebacterium glutamicum* containing the gene for alpha-1,2-fucosyltransferase from *Corynebacterium urelyticum*".

There is no change to:

- "S3—51 Specification 2'-fucosyllactose sourced from *Corynebacterium glutamicum*", since the strain is not currently specified in this Schedule.
- the uses and levels of 2'-FL in "Standard 2.9.1 Infant Formula Products". The intended use levels are consistent with the permitted levels for 2'-FL in Schedule 29 (96 mg/100 kJ, equivalent to 2.4 g/L) (Schedule S29-54, Infant formula products – substances permitted as nutritive substances)

The approval will provide an additional/alternative source of the Human Milk-identical Oligosaccharide 2'-Fucosyllactose (2'-FL) for use in infant formula products, under the controls already laid down in Standard 2.9.1 Infants Formula Products.

This source will enable alternative suppliers and therefore increase competition to keep raw materials costs as low as possible to provide economic benefits to the consumer.

Corynebacterium glutamicum (*C. glutamicum*) has been granted Qualified Presumption of Safety (QPS) status in the European Union (EU) by EFSA and therefore is considered safe for the derivation of genetically modified (GM) strain lineages intended for use in the production of food additives and enzymes. The parental strain was engineered in a targeted manner to over-express the 2'-FL synthesis pathway and to produce higher yields of 2'-FL. General strain engineering methods were used to introduce genetic modifications like gene deletions and insertions into the production strain genome. The final production strain is plasmid-free and antibiotic marker free and is stable at the phenotype and genotype level. The strain has been deposited and

registered in an international culture collection centre. The whole genome sequence has been provided for the host strain and production strain and confirmed taxonomic identity and intended genomic modifications.. The absence of viable production microorganism and of its GM DNA in the 2'-FL product has been demonstrated.

The production process is similar to all other approved microbial sources of 2'-FL.

The first stage, upstream processing (USP), is fermentation for the production of 2'-FL in a stirred type reactor (STR). Fermentation of the production organism is performed in a complex medium including yeast extract, soy peptone and trace elements with well controlled process parameters (e.g., temperature, pH, dissolved oxygen (DO), aeration). Glucose or sucrose is fed continuously as a carbon source and lactose is also fed as substrate for cells to synthesize 2'-FL. The 2'-FL ingredient is excreted into the media. At the end of the fermentation step, the production organism is inactivated by thermal treatment and removed by microfiltration.

In a second stage, after biomass removal, the fermentation broth undergoes a series of downstream purification processes (DSP) to purify 2'-FL by removing impurities sequentially and followed by drying steps. First, large molecules, mainly proteins, are removed by ultrafiltration, followed by a further concentration step (nanofiltration, evaporation) to reduce the volume. Next, active charcoal is applied to remove colour and organic matter, followed by electrodialysis and ion exchange column to remove charged inorganic salts. Next, saccharide impurities are removed by chromatography, then followed by spray drying to obtain the final 2'-FL ingredient.

All raw materials, processing aids (input materials) and food contact materials are food grade, and in accordance with applicable regulations.

The manufacturing process for 2'-FL is controlled by current Good Manufacturing Practices (cGMP) and the principles of Hazard Analysis and Critical Control Points (HACCP) plan. Critical Control Points (CCPs) are identified, and in-process controls are implemented throughout the process based on the HACCP plan. Raw materials and processing aids are food-grade, and packaging materials are permitted for food contact use. Master operating instructions are followed, all records are kept, and the final 2'-FL product is controlled and documented by production of a Certificate of Analysis and lot release procedures.

Identity testing using nuclear magnetic resonance (1 H-NMR, 13C-NMR and 2D NMR) spectroscopy confirms "2'-fucosyllactose from the source organism, *Corynebacterium glutamicum* containing the gene for alpha-1,2-fucosyltransferase from *Corynebacterium urelyticum*" to be chemically and structurally equivalent to 2'-FL in human milk. Additionally, each batch of the applicant's 2'-FL was compared to the 2'-FL standard using liquid chromatography with mass spectrometry (LC-MS/MS) to confirm the identity.

Compositional data on 5 production representative batches demonstrates that 2'-FL is manufactured in a consistent manner and demonstrates the absence of process-related and undesirable substances at levels of safety concern. Stability testing demonstrates the absence of deterioration of the physico-chemical and microbiological profile under normal storage conditions.

Cataya has conducted a series of toxicological tests to support the safety, including "tier 1" requirements:

- Acute oral toxicity test report – "non-toxic" lethal dose (LD)50 value of at least 10.0 g/kg
- In vitro Bacterial reverse mutation test - negative
- In vitro mammalian chromosome aberration test - negative
- In vivo mammalian erythrocyte micronucleus test - negative
- Repeated dose 90-day oral toxicity test in the rat - the no observed adverse effect level (NOAEL) for the repeated dose 90-day oral toxicity test of 2'-FL in female and male rats were determined to be 9.16 and 7.43 g/kg bw/day, respectively
- Teratogenicity test in the rat - the NOAEL of 2'-FL on pregnant rats and foetal rats is ≥10.0 g/kg bodyweight/day.

There is no change to the nutritional properties of 2'-FL.

The host species *C. glutamicum* is considered as QPS by EFSA and therefore does not give rise to inherent concern of allergenicity. Cataya conducted a bioinformatic allergenicity assessment of the inserted DNA sequences in the 2'-FL production strain using FAO/WHO-recommended methods, which indicated a low potential for allergenicity.

In conclusion "2'-fucosyllactose from the source organism, *Corynebacterium glutamicum* containing the gene for alpha-1,2-fucosyltransferase from *Corynebacterium urelyticum*" is safe and suitable for existing approved uses of 2'-FL.