EXECUTIVE SUMMARY: APPLICATION TO AMEND THE *AUSTRALIA AND NEW ZEALAND FOOD STANDARDS CODE* TO ALLOW FOR THE USE OF 2'-FUCOSYLLACTOSE PRODUCED USING GENE TECHNOLOGY FOR USE AS A NUTRITIVE SUBSTANCE IN INFANT FORMULA PRODUCTS

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The nutritive substance that is the subject of this application is 2'-fucosyllactose (2'-FL), which is produced using a modified strain of *Escherichia coli* W as a processing aid. 2'-FL is a fucosylated oligosaccharide composed of 3 monosaccharides, namely L-fucose, D-galactose, and D glucose.

2'-FL is currently permitted for use as an ingredient in infant formula products under Schedule 29-5 of the *Australia New Zealand Food Standards Code* (the Code) Section 2.9.1-5 (Infant Formula Products – substances permitted for use as nutritive substances), with permitted source organisms including modified strains of *E. coli* K-12 and BL21. Kyowa is seeking to amend Schedules 3, 26, and 29 of the Code to include their 2'-FL produced using a modified strain of *E. coli* W. Kyowa is not seeking to amend the currently permitted uses or use levels of 2'-FL.

The host organism used in the construction of Kyowa's production strain, *E. coli* W, is well-characterised and is 1 of 4 *E. coli* strains designated as Risk Group 1 organisms in biological safety guidelines, as it does not cause disease in healthy adult humans, and does not colonise the human gut. Evaluation of the production strain according to the European Food Safety Authority's (EFSA's) *Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use* indicates no safety concerns resulting from the genetic modifications.

Kyowa's 2'-FL produced by microbial fermentation using a genetically modified strain of *E. coli* W is manufactured using food-grade raw materials and processing aids and is conducted in accordance with a detailed Hazard Analysis and Critical Control Points (HACCP) plan. The production microorganism is cultured in chemically defined nutrient media under sterile conditions in tightly controlled conditions. In the main fermentation process, the production microorganism synthesises 2'-FL, which is excreted into the media. 2'-FL is isolated and purified from the fermentation medium using a series of filtration and cationic and anionic exchange steps, followed by concentration and spray drying to obtain the final 2'-FL product.

Kyowa's 2'-FL has been demonstrated by high-performance liquid chromatography with pulsed amperometric detection, proton nuclear magnetic resonance spectroscopy, and carbon-13 nuclear magnetic resonance spectroscopy to be structurally and chemically identical to 2'-FL that is naturally present in human breast milk. Kyowa's purity limit for 2'-FL (≥82%) is similar to 2'-FL ingredients authorised on EFSA's Union list of novel foods (≥83% to ≥95%) and permitted for use in the Code. Minor differences in the carbohydrate profile of Kyowa's 2'-FL ingredient from those currently permitted do not pose a safety concern, as the carbohydrates are all naturally occurring in human breast milk or are human breakdown products of naturally occurring components of human milk, and exposures to these carbohydrates from the intended uses of Kyowa's 2'-FL are expected to be insignificant compared to background exposures.

Analytical data on Kyowa's 2'-FL ingredient demonstrate that the use of Kyowa's modified *E. coli* W-derived production strain as a processing aid does not adversely affect the composition or the level of undesirable substances in the final ingredient.

The safety and suitability of 2'-FL has been assessed previously by Food Standards Australia New Zealand (FSANZ), with no evidence of safety or nutritional concerns identified. In a literature search conducted by Kyowa to identify any information relevant to the safety of 2'-FL published since the most recent FSANZ evaluation of 2'-FL (June 2021), no studies were identified that indicated the potential for allergic, toxic, or adverse health effects related to consumption of 2'-FL.

Toxicology studies of Kyowa's 2'-FL ingredient were conducted to satisfy the requirements of other jurisdictions. Kyowa's 2'-FL ingredient was not mutagenic in a bacterial reverse mutation assay or in an *in vivo* micronucleus study. In a 90-day toxicity study, there were no toxicologically relevant compound-related adverse effects reported, and the study authors determined the no-observed-adverse-effect level to be 2,000 mg/kg body weight/day, the highest dose tested. The results of these studies support the safety of Kyowa's 2'-FL ingredient. Considering that Kyowa's 2'-FL ingredient is chemically and compositionally similar to other 2'-FL ingredients currently authorized in the Code, the existing safety conclusions by FSANZ on these other 2'-FL ingredients may be extended to support the safety of Kyowa's 2'-FL produced using a genetically modified strain of *E. coli* W.

The results of recently published studies of other 2'-FL ingredients further support the safety of Kyowa's 2'-FL under the intended conditions of use and are in agreement with the pre-clinical and human studies of 2'-FL previously evaluated by FSANZ. The minor differences in specifications between Kyowa's 2'-FL and those permitted in the Code do not affect the nutritional value, metabolism, or level of undesirable substances in the ingredient, and therefore, do not affect the results of the existing risk assessment for 2'-FL as currently included in the Code.

The totality of the available data on 2'-FL produced using a genetically modified strain of *E. coli* W supports the safe use of the ingredient under the conditions of use currently permitted in the Code.