# APPLICATION TO AMEND THE SPECIFICATIONS FOR LACTO-*N*-TETRAOSE (LNT) UNDER THE *AUSTRALIA AND NEW ZEALAND FOOD STANDARDS CODE*

## EXECUTIVE SUMMARY

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# EXECUTIVE SUMMARY

FrieslandCampina Ingredients B.V. (hereinafter "FrieslandCampina) is submitting an application to Food Standards Australia New Zealand (FSANZ) with regard to a purified human milk oligosaccharide (HMO) ingredient, lacto-*N*-tetraose (LNT), which is produced using a proprietary strain of genetically engineered *Escherichia coli* (derived from strain K-12). The applicant is therefore seeking approval for the use of this ingredient in infant formula products as a nutritive substance, at use levels that are equivalent to those that are already permitted for other LNT ingredients.

FrieslandCampina has developed the technology to enable the production of LNT, *via* fermentation, utilising a proprietary strain of *Escherichia coli* (derived from *E. coli* strain K-12) that has been genetically engineered to contain the genes for beta-1,3-*N*-acetylglucosaminyltransferase from *Neisseria meningitides* and the gene for beta-1,3-galactosyltransferase from *Helicobacter pylori*. In Australia and New Zealand, LNT produced from fermentation is already permitted for use as a nutritive substance in infant formula products in accordance with *Schedule 3 – Identity and purity* and *Schedule 26 – Food produced using gene technology* of the *Australia New Zealand Food Standards Code* ("the Code") when it is sourced from genetically engineered *E. coli* K-12 containing the genes for β-1,3-*N*-acetylglucosaminyltransferase from *N. meningitides* and β-1,4-galactosyltransferase from *H. pylori*. As such, the microbial source employed by FrieslandCampina in the manufacture of this LNT ingredient is already permitted for such use in Australia and New Zealand; however, the defined specifications for the identity and purity of LNT (*i.e.*, S3—48) currently precludes the use of other LNT preparations that could otherwise meet all other regulatory requirements. Specifically, FrieslandCampina's LNT ingredient meets or exceeds the ≥70% LNT purity criteria but also includes a wider acceptable pH range and a modified profile of minor carbohydrates from what is currently defined in Schedule 3. These differences are due to the inherent variation that exists between different proprietary microbial sources (such as codon optimisation) and do not introduce any potential concern related to safety. The applicant therefore aims to amend Schedule 3 of the Code to include a new (or modified) list of specifications, to encompass the specifications that have been established for FrieslandCampina's LNT.

The proposed change to amend Schedule 3 *via* inclusion of a new (or modified) set of specifications for the identity and purity of LNT represents an opportunity to introduce an alternative ingredient to the Australia and New Zealand marketplace from what is currently permissible (and does not include any modifications to the currently permitted uses or use levels of this ingredient). The availability and use of alternative LNT ingredient is expected to promote an efficient and internationally competitive food industry, which would thereby benefit the Australia and New Zealand consumer, in addition to facilitating harmonisation with domestic and international food standards.

Production of the FrieslandCampina's LNT is conducted in accordance with current Good Manufacturing Practice and the principles of Hazard Analysis and Critical Points (implemented *via* in-process control and critical control points). The manufacturing process of LNT involves the fermentation of glucose and lactose with a genetically modified strain of *E. coli*, which is conducted in sterilised fermentation equipment containing a minimalistic medium and no added complex organic substances. LNT is excreted into the fermentation media where it then undergoes a series of downstream processing steps to purify and concentrate the ingredient. The final LNT ingredient is free of the production organism and contains a minimum of 75% LNT as the primary constituent with minor amounts of other related carbohydrates. All raw materials, processing aids, and food contact articles used in the production of LNT are food-grade or better, are not major allergens, and are used in accordance with applicable regulations.

Product specifications for FrieslandCampina's LNT, as presented within this application, have been established to align with those that have been presented to the United States (U.S.) Food and Drug Administration and the European Food Safety Authority in parallel regulatory applications. Appropriate specifications for chemical purity parameters, composition, microbial and heavy metal contaminants, and endotoxins have been defined for this ingredient. As described above, many of these specification parameters are already in compliance with those that are currently defined for LNT in Schedule 3 of the

Code. Results from batch analyses of 5 non-consecutive lots of FrieslandCampina's LNT, conducted with internationally recognised methods or validated internal analytical methods, demonstrate that the manufacturing process produces a consistent product that meets defined specifications.

LNT has a long-established history of safe consumption in the human diet as a component of human breast milk, and more recently, as an ingredient in infant formula products. FrieslandCampina's LNT has been analytically demonstrated to be chemically and structurally identical to the LNT in human milk and can therefore be expected to undergo the same metabolic pathways as its naturally occurring counterpart. LNT does not undergo significant digestion in the upper gastrointestinal tract. The safety of the applicant's LNT has been assessed in a range of product-specific toxicity studies including a bacterial reverse mutation assay, an *in vitro* mammalian cell chromosome aberration test, *in vitro* and *in vivo* mammalian cell micronucleus tests, and a 90-day repeat-dose oral toxicity study conducted in rats. The safety of several other LNT preparations, produced from microbial fermentation, has also been extensively evaluated by FSANZ and other scientific bodies and regulatory agencies such as the U.S. Food and Drug Administration (FDA) and the European Food Safety Authority. An updated comprehensive search of the published scientific literature was conducted to identify any newly published scientific articles that were not evaluated in the most recently gazetted FSANZ application for LNT produced by microbial fermentation (*i.e.,* A1265). No new data were identified that could contradict the original conclusions of safety for LNT.

Additional considerations were made with regards to the safety of the production microorganism (*i.e.*, a genetically engineered *E. coli*, derived from strain K-12). This production organism is derived from the non-pathogenic strain *E. coli* K-12—an organism with a time-proven safety record of over approximately 100 years of use in the laboratory and 40 years use in commercial fermentations. Moreover, genetically engineered *E. coli* K-12 is already employed in the manufacture of LNT for use in infant formulae in Australia and New Zealand. To evaluate the potential allergenicity of proteins encoded by genes required for LNT biosynthesis, a series of bioinformatic searches were conducted, indicating a lack of potential for allergenicc cross-reactivity. Additionally, results from the analyses of the Applicant's LNT confirmed the absence of residual DNA and viable cells of the production organism in the final ingredient, confirming that there is no safety concern related to the production organism with this ingredient.

LNT, which plays a crucial role in infant health and development, including prebiotic effects, antiadhesive antimicrobials, antiviral protection, and immune modulators, is currently as a nutritive substance in infant formula products in Australia and New Zealand. FrieslandCampina's ingredient is proposed for use under the same conditions of use as those presently authorised for LNT and is expected to be fully substitutional to the LNT that is currently marketed. The anticipated intakes of this ingredient are therefore unlikely to change from the current levels of intake following a successful application. Since FSANZ previously conducted a safety review LNT from microbial fermentation, a separate intake assessment for FrieslandCampina's LNT was not performed for this application; however, intakes estimates conducted for the U.S. and European populations (from parallel regulatory submissions in other jurisdictions) were provided instead. As the current application does not propose an extension of use, additional considerations pertaining to the nutritional impact of LNT and its impact on consumer understanding and behaviour was not included.

The totality of evidence provided in this application supports the safe use of FrieslandCampina's LNT as a nutritive substance in infant formula products, at currently permitted levels of use, in Australia and New Zealand. The microbial source used by FrieslandCampina to obtain LNT (a genetically engineered *E. coli* K-12) has already gained approval for use in Australia and New Zealand. FrieslandCampina's LNT has been demonstrated to be structurally and chemically identical to the LNT naturally present in human breast milk and can therefore be expected to undergo the same metabolic pathways. An extensive safety database exists for LNT, which corroborates the conclusions of safety established for FrieslandCampina's LNT from the results of product-specific safety data for this ingredient. The weight of the scientific evidence presented in this application indicates that the proposed amendment of the Code, to include a new or modified list of specifications to permit FrieslandCampina's LNT as alternative form of what is currently marketed, does not present a significant risk to human health.