Public summary

Novel Food application for Lacto-N-Tetraose (LNT)

Applicant: Inbiose N.V., Technologiepark 82, bus 41, B-9052 Zwijnaarde, Belgium

The subject of this novel food application is Lacto-N-Tetraose (LNT) that is manufactured by fermentation with an engineered strain of *E. coli*, followed by a sequence of isolation and purification steps.

LNT is a neutral human milk-oligosaccharide (HMO) that is abundant in breast milk. The tetrasaccharide consists of a molecule of D-galactose linked to a N-acetyl-D-glucosamine (**β**-(1-3) bond), linked to D-galactose (**β**-(1-3) bond), linked to the reducing end D-glucose (**β**-(1-4) bond),

In 2020, a novel food authorization for LNT was granted according to Article 26 of Regulation (European Commission, 2020 amended by European Commission, 2021; European Commission, 2017). Therefore, INBIOSE requests an own authorization for LNT, which requires the specifications in the union list to be adapted.

The present application was prepared pursuant with the most recent EFSA guidance for applicants (EFSA, 2021) and the Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA, 2018).

The novel food is manufactured using a genetically modified strain of *E. coli* K12 MG1655, which is quantitatively removed from the product. A sequence of filtration and chromatography steps results in LNT with a purity of not less than 80 % w/w on dry matter. The total concentration of human milk saccharides is not less than 90 % w/w on dry matter, including lactose and Lacto-N-Triose II. The identity of LNT has been unequivocally demonstrated by ^{1}C and ^{13}HNMR. The applicant demonstrated in five independently manufactured product batches that the novel food meets the specifications with respect to purity, and the provisions laid down in Regulations (EC) 1881/2006 and (EC) 2073/2005 concerning chemical or microbial contaminations. The stability studies with Inbiose´s LNT powder under normal and accelerated conditions demonstrated that LNT is stable with no measurable change in the content of LNT up to 18 months. The applicant aims at an extended shelf life of this novel food. Therefore, the stability study is set up for a period of 5 years at real time conditions. As demonstrated in model food applications, LNT is also stable under typical food processing conditions over periods of time that are relevant for these applications.

The production process including the generation of the production strain, the fermentation process and the downstream processing have been described in detail. The safety of the production strain is supported by WGS data and the history of safe use of the host

microorganism *E. coli* K-12 MG1655. The final product does not contain viable cells or recombinant DNA resulting from the production strain.

The safety of LNT manufactured by INBIOSE has been verified in a series of toxicity studies, including a bacterial reverse mutation assay, an *in-vitro* micronucleus assay, and a 90-day oral toxicity study in juvenile rats with dose-range finding study. None of the studies gave rise to substance-related safety concerns.

LNT is intended for the general population including infants except for food supplements, for which the target population is individuals above 12 months of age. Proposed uses and use levels for LNT are the same as already authorized by (EU) 2020/484 (European Commission, 2020 amended by European Commission, 2021; European Commission, 2017).

The data presented in this novel food application support the safety and suitability of LNT for the proposed uses.

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