Summary of the dossier: Lacto-*N*-neotetraose (LNnT)

Applicant: Jennewein Biotechnologie GmbH, Maarweg 32 53619 Rheinbreitbach, Germany

This application concerns the extension of the present specifications for Lacto-*N*-neotetraose (LNnT) from microbial sources, more specifically for including L*N*nT manufactured by fermentation with a metabolically engineered strain *E. coli* BL21 (DE3) into the Union List of Novel Foods (EU) 2018/1023.

The application has been compiled in line with the administrative and scientific requirements of Commission Implementing Regulation (EU) 2017/2469 laying down for applications referred to in Article 10 of Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods. It is also in line with the European Food Safety Authority (EFSA) guidance on the preparation and presentation of an application for authorisation of a Novel Food in the Context of Regulation (EU) 2015/2283.

L*N*nT is a human milk oligosaccharide (HMO). HMOs are constituents in breast milk that play an important role in the healthy development of infants by shaping the gut microbiota, positively influencing the brain development and forming a healthy immune system of infants. Since 2015, two HMOs have been authorized as novel foods, namely 2'-fucosyllactose (2'-FL) and L*N*nT. More novel food applications for HMOs manufactured by fermentation have been submitted.

According to the current Union List of Novel Foods (EU) 2018/1023, L*N*nT can either be manufactured by chemical synthesis or fermentation with a metabolically engineered strain of *E. coli* K-12. The present application concerns the inclusion of L*N*nT that is manufactured using a metabolically engineered strain of *E. coli* BL21 (DE3). The applicant used the same host strain to manufacture 2'-FL, which was authorized as novel food in 2017. The manufacturing process consists of the fermentative production of L*N*nT by the bacteria, followed by a sequence of purification steps. The L*N*nT produced by this process is a white, easily water-soluble powder. The identity of the L*N*nT was verified by ^{1}H and ^{14}C NMR and LC-MS/MS.

The purity criteria of the L*N*nT correspond to the specifications in the Union List of Novel Foods (EU) 2018/1023, with a minimally reduced L*N*nT content (90 % instead of 92 %), and a slightly higher ash content (1 % instead of 0.4 %). The product preparation process completely removes the production strain and any components of the bacterial cells, as verified by microbial analyses, protein content determination and PCR.

The conditions of use and use levels for the L*N*nT in the present application are exactly the same as laid down in the Union List (EU) 2018/1023 for the already authorized L*N*nT from a microbial process with a metabolically engineered strain of *E. coli* K-12. The proposed amendment to the specifications in the Union List would read as follows:

Definition: Chemical Name: β-D-Galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-D-glucopyranose, Chemical formal: C26H45NO21 CAS No: 13007-32-4 Molecular weight: 707.63 g/mol Source: Genetically modified strain of *Escherichia coli* BL21 (DE3) Description: Lacto-*N*-neotetraose is a white to off-white powder that is produced by a microbiological process. Purity: Assay (water free): ≥ 90 % D-Lactose: ≤ 3.0 % Lacto-*N*-triose II: ≤ 3.0 % para-Lacto-*N*-neohexaose: ≤ 3.0 % pH (20 °C, 5 % solution): 4.0-7.0 Water: ≤ 9.0 % Ash: ≤ 1.0 % Residual proteins ≤

0.01 % Microbiological criteria: Aerobic mesophilic bacteria total count: ≤ 10000 CFU/g Yeasts and moulds: ≤ 100 CFU/g Residual endotoxins: ≤ 10 EU/mg