SUBSTANTIAL EQUIVALENCE OPINION

Lacto-*N*-neotetraose produced by microbial fermentation (LNnT)

The Food Safety Authority of Ireland (FSAI) received an application in May of 2016 from Glycom A/S in Denmark for an opinion on the substantial equivalence of lacto-*N*-neotetraose (LNnT) produced by microbial fermentation to a chemically synthesised version of the same ingredient which was authorised for the EU market through Commission Implementing Decision 2016/375. Glycom intends the novel ingredient to be used in infant formula, follow-on formula, other foods for infants and young children, food supplements and a variety of general food categories as set out in *Annex II* of Commission Implementing Decision 2016/375.

The novel ingredient is a tetrasaccharide (D-galactose, *N*-acetyl-D-glucosamine, D-galactose and D-glucose) and is chemically and structurally identical to the same tetrasaccharide naturally found in some mammalian milk, the highest concentrations being found in human milk. It is produced by a fermentation process using a genetically modified *E. coli* K12 strain. This bacterial strain is considered a processing aid because the tetrasaccharide is secreted during fermentation, the microbial cells are not disrupted and are absent from the final product. Therefore, approval and labelling of the novel ingredient under EU GM food & feed legislation is not required.

The applicant considers that LNnT produced by microbial fermentation falls within the category of "*foods and food ingredients consisting of or isolated from microorganisms, fungi or algae"* as described in *Article 1 (d)* of the novel food Regulation EC No 258/97.

Composition

The applicant has used 1D and 2D methods of ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) along with mass spectrometry to confirm the chemical and structural identity of LNnT produced by fermentation. It is chemically and structurally identical to the synthetic LNnT (CAS Registry Number 13007-32-4) as well as the LNnT naturally occurring in human milk. The specifications for the fermentation product are very similar to the authorised synthetic counterpart, with any of the differences being considered minor in terms of the overall purity. A slight reduction in the LNnT assay value is proposed by the applicant to be due to the presence of oligosaccharides including lactose, lacto-*N*-triose II and *para*-lacto-*N*-

neohexaose that are also naturally present in human milk, but formed at slightly higher levels in the fermentation process compared to synthetic process.

Parameter	**LNnT Produced by Chemical Synthesis**	**LNnT Produced by Fermentation**
	Specification	Specification
Identification[1]	= RT of standard ± 3 %	= RT of standard ± 3 %
Human-identical Milk Saccharides[2]	Not less than 96 %	Not less than 95 %
Assay LNnT (water free)	Not less than 96 %	Not less than 92 %
D-Lactose	Not more than 1.0 %	Not more than 3.0 %
Lacto-*N*-triose II	Not more than 0.3 %	Not more than 3.0 %
para-lacto-*N*-neohexaose	Not applicable[3]	Not more than 3.0 %
LNnT fructose isomer	Not more than 0.6 %	Not more than 1.0 %
pH (20°C, 5% solution)	5.0 to 7.0	4.0 to 7.0
Water	Not more than 9.0 %	Not more than 9.0 %
Ash, sulphated	Not more than 0.4 %	Not more than 0.4 %
Acetic acid	Not more than 0.3 %	Not applicable
Residual solvents (methanol, 2-propanol, methyl acetate, acetone)	Not more than 50 mg/kg singly and not more than 200 mg/kg in combination	Not more than 100 mg/kg methanol
Residual proteins	Max. 0.01 %	Max. 0.01 %
Palladium	Max. 0.1 mg/kg	Not applicable
Nickel	Max. 3.0 mg/kg	Not applicable

RT = retention time.

[1] Specification not used in regulatory filing of LNnT produced by chemical synthesis but applied.

[2] Human-identical Milk Saccharides = Sum of LNnT, D-Lactose, Lacto-*N*-triose II, and *para*-lacto-*N*-neohexaose. Specification not used in regulatory filing of LNnT produced by chemical synthesis but applied.

[3] This naturally occurring hexasaccharide of human milk is not a possible by-product of the LNnT chemical synthesis

Nutritional Value and Metabolism

The chemical and structural identity of LNnT is the same regardless of whether it is naturally occurring, synthetic or produced by microbial fermentation. Therefore the nutritional value and metabolism of LNnT should be the same regardless of the source.

With the exception of zinc, trace elements were not detected in the novel ingredient as might be expected through carry-over from the fermentation process. The results show that zinc may be present at levels of 0.2-0.4 mg/kg of the final product. Taking into consideration the predicted worst-case scenario calculated for the synthetic comparator (95th percentile intake

of 3.32 g/day of LNnT for women of child-bearing age), this level of zinc would contribute only 0.013% to the daily reference intake of 10 mg/day and so is not a concern.

Intended uses

The novel ingredient is intended for use in the same food categories and at the same maximum levels as the authorised synthetic counterpart and which are set out in *Annex II* of Commission Implementing Decision (EU) 2016/375. Food categories include infant and follow-on formula, other foods for infants and young children, dietary foods for special medical purposes and food supplements (except food supplements for infants). General foodstuffs to which the novel ingredient will be added include milk based products, dairy analogues, cereal bars, table-top sweeteners and beverages.

Level of Undesirable Substances

Because of the different production methods, the profile of undesirable substances in the novel LNnT is somewhat different to the synthetic counterpart. The novel LNnT is produced by fermentation using a metabolically engineered strain of *Escherichia coli* K12. LNnT is secreted extracellularly during the fermentation process and the bacteria ultimately removed by ultra-filtration upon completion of the fermentation process. Methanol is the only solvent used in the production of the novel ingredient, where it functions as a crystallisation aid in the downstream processing stages. Batch analysis results for the final product are provided which demonstrate that the presence of methanol is reproducibly well within the specification set down by the applicant. Microbial contaminants including *Cronobacter sakazakii*, *L. monocytogenes*, *B. cereus*, *E.coli* and *Salmonella* are all within the applicant's specification and equivalent to those for the synthetic comparator. The absence of endotoxins is also demonstrated.

To provide further reassurance about the safety of the novel ingredient, the applicant carried out preclinical toxicology studies, similar to those presented in support of the authorisation of the synthetic comparator. These studies included an adapted subchronic 90-day oral toxicity study in rats, a bacterial reverse mutation assay and an *in vitro* micronucleus assay.

Conclusions

The FSAI is satisfied from the information provided that lacto-*N*-neotetraose (LNnT) produced by Glycom A/S using microbial fermentation is substantially equivalent to the synthetic counterpart authorised for the EU market by Commission Implementing Decision

(EU) 2016/375 in terms of composition, nutritional value, metabolism, intended use and level of undesirable substances.

In accordance with *Article 2* of Commission Implementing Decision (EU) 2016/375; the designation of this ingredient in foods containing it will be "lacto-*N*-neotetraose"; Information shall be provided to consumers that supplements containing the novel ingredient should not be used if other foods with added LNnT are consumed on the same day; Information shall be provided to consumers that supplements containing the novel ingredient and intended for young children should not be used if breast milk or other foods with added LNnT are consumed on the same day.