

Assessment of Lacto-N-tetraose (LNT) as a Novel Food for Use in Food and Food Supplements (RP2101)

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Keywords: Regulated products, Novel foods, Safety assessment

<https://doi.org/10.46756/001c.124201>

FSA Research and Evidence

Chr. Hansen, Denmark (“the applicant”) submitted a novel food application for the authorisation of lacto-N-tetraose (LNT) as a novel food to each nation of Great Britain in September 2023.

The novel food is intended to be used as a source of human identical milk oligosaccharide, LNT, and is manufactured by microbial fermentation using a genetically modified strain of *Escherichia coli* BL21 (DE3), and then refined to yield the purified powder.

This new application is seeking to use the novel food within the following food categories: food for special medical purposes and food supplements for the general population including those for vulnerable groups (pregnant and breastfeeding women, and the elderly); food for infants and young children including infant formula and follow-on formula. Food supplements are not intended to be used if other foods with added LNT or breast milk are consumed the same day.

This novel food had its application for authorisation assessed by the European Food Safety Authority (EFSA) which was published in March 2022. The Food Standards Agency (FSA) and Food Standards Scotland (FSS) have reviewed the information available, including the EFSA opinion, and confirmed that LNT is safe under the proposed conditions of use. The anticipated intake levels and proposed use in food and food supplements was not considered to be nutritionally disadvantageous.

This safety assessment represents the opinion of the FSA and FSS.

1. Introduction

In accordance with assimilated Regulation (EU) 2015/2283 on novel foods, the application RP2101 for the use of lacto-N-tetraose (LNT) as a novel food, has been submitted for authorisation in each nation of Great Britain (GB).

Whilst it was a Member State of the EU, the UK accepted the risk assessments of the European Food Safety Authority (EFSA) in respect of authorisations for regulated food and feed products. Since the end of the transition period, FSA and FSS have adopted equivalent technical guidance and quality assurance processes to be able to undertake GB risk assessments for regulated product applications.

To ensure our regulatory systems are risk proportionate and resources are used effectively, the FSA and FSS have used the evidence submitted by the applicant and other information in the public domain, including the EFSA risk assessment opinion, to inform this safety assessment.

The FSA and FSS reviewers have evaluated the published EFSA risk assessment on the novel food and confirmed that this is appropriate for GB risk analysis. Consideration has been given to the processes undertaken to ensure the EFSA opinion is robust and whether there are any aspects that would require further review, such as specific issues for the countries of GB. The result of the assessment is that there is sufficient evidence of safety to conclude without requiring further risk assessment at this time.

This safety assessment represents the opinion of the FSA and FSS.

2. Details of other regulators opinions

The applicant, Chr. Hansen A/S (Denmark), is seeking authorisation for LNT as novel food produced by fermentation using genetically modified *E. coli* BL21 (DE3).

The first novel food application for LNT, which was submitted by Glycom A/S (Denmark), is produced by fermentation using genetically modified *E. coli* K-12 DH1. A positive EFSA opinion was published in October 2019 (EFSA NDA Panel, 2019). This novel food is currently authorised in GB (and listed in assimilated Commission Implementing Regulation (EU) 2017/2470) for use in the following food categories: dairy products and analogues, bakery wares, beverages, foods for special groups, and food supplements.

LNT produced by fermentation using genetically modified *E. coli* BL21 (DE3) (Jennewein Biotechnologie GmbH, Germany – wholly owned by Chr. Hansen A/S, Denmark) was notified to the FDA (Food and Drug Administration) in the USA through their Generally Regarded as Safe (GRAS) scheme (FDA, 2021) and is permitted to use as an ingredient in infant formula only. A second application in August 2022 for LNT produced using the same genetically modified organism, is GRAS (FDA, 2022) as a food ingredient in foods for special groups and beverages.

In March 2022, the safety of LNT produced by fermentation using genetically modified *E. coli* BL21 (DE3) (Chr. Hansen A/S, Denmark), was assessed by EFSA and received a positive opinion (EFSA NDA Panel, 2022). This opinion has been reviewed by the FSA and FSS risk assessors.

2.1. Methodology applied in the EFSA opinion

EFSA conducted the assessment of the novel food in accordance with the procedure as outlined in the EFSA scientific opinion 'Guidance on the preparation and submission of an application for authorisation of a novel food in the context of Regulation (EU) 2015/2283 (Revision 1)' (EFSA NDA Panel, 2021) and Commission Implementing Regulation (EU) 2017/2469.

2.1.1. Identity of the novel food

The novel food is a purified white to ivory powder containing $\geq 75\%$ LNT by dry weight. Other saccharides are present in smaller quantities: D-lactose ($\leq 5\%$ w/w dry weight), D-galactose and D-glucose ($\leq 5\%$ w/w dry weight, sum of both), *para*-lacto-N-hexaose ($\leq 5\%$ w/w dry weight), lacto-N-triose II ($\leq 5\%$ w/w dry weight) and a small fraction of other related saccharides (sum of other carbohydrates $\leq 15\%$ w/w dry weight).

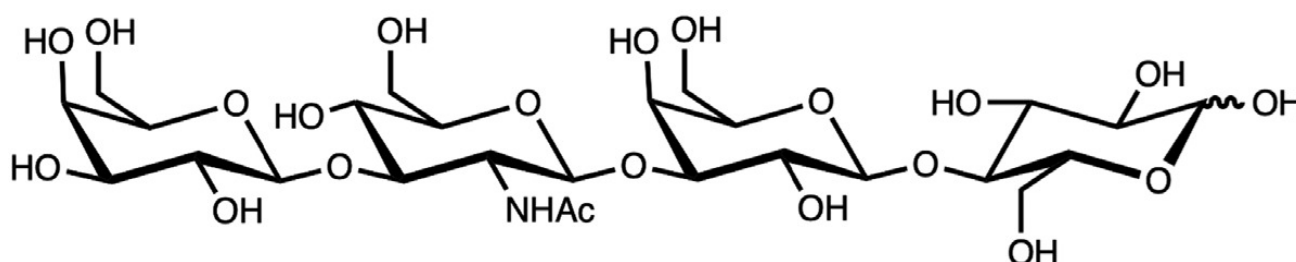


Figure 1. The structural formula of lacto-N-tetraose (LNT).

LNT is a tetrasaccharide consisting of D-galactose, N-acetyl-D-glucosamine, D-galactose and D-glucose (see [Figure 1](#)) where the D-galactose is linked through a β -(1-3) bond to N-acetyl-D-glucosamine, N-acetyl-D-glucosamine is linked through a β -(1-3) bond to D-galactose, and D-galactose is linked through a β -(1-4) bond to the reducing end of D-glucose, in its α - and β -anomeric forms.

LNT is a regioisomer of lacto-N-neotetraose (LNnT). Both isomers contain the same monosaccharide moieties but with the linkage between the terminal D-galactose and N-acetyl-D-glucosamine being β -(1-4) rather than β -(1-3).

LNT is characterised by the following information:

- IUPAC name:
N-[(2S,3R,4R,5S,6R)-2-[(2R,3S,4S,5R,6S)-3,5-dihydroxy-2-(hydroxymethyl)-6-[(2R,3S,4R,5R)-4,5,6-trihydroxy-2-

(hydroxymethyl)oxan-3-yl]oxyoxan-4-yl]oxy-5-hydroxy-6-(hydroxymethyl)-4-[(2R,3R,4S,5R,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxyoxan-3-yl]acetamide

- CAS number: 14116-68-8
- Molecular formula: C₂₆H₄₅NO₂₁
- Molecular mass: 707.63 g/mol

The molecular structure of the LNT in the novel food was determined by high performance liquid chromatography – mass spectrometry (HPLC-MS). The collision induced decay (CID) fragmentation patterns from the LNT in the novel food were compared with a high purity in-house standard and a commercially available substance standard.

The identity of the novel food was also confirmed by high-performance anion-exchange chromatography – pulsed amperometric detection (HPAEC-PAD) with a high purity in-house standard.

Confirmation that the LNT in the novel food, is equivalent to LNT found in human breast milk was provided by comparative nuclear magnetic resonance (NMR) spectroscopy: double-quantum filtered ¹H¹H-correlated spectroscopy (COSY) NMR, phase-sensitive ¹H¹³C-heteronuclear single quantum correlation (HSQC) NMR and phase-sensitive ¹H¹³C-heteronuclear multiple bond correlation (HMBC) NMR.

2.1.2. Production process

The novel food is manufactured using a two-step batch fermentation process. In step 1, the raw materials, lactose and glycerol (glucose and sucrose can be used as alternatives to glycerol) are converted into LNT using a genetically modified microorganism (GMM) derived from *E. coli* BL21 (DE3). A second GMM, derived from the same *E. coli* strain, can also be used to breakdown saccharide by-products and any remaining substrates. In step 2, a series of purification and isolation steps are used to concentrate and purify the novel food. The novel food is then spray dried to a powder containing ≥ 75% LNT by dry weight.

Information on the hazard identification, hazard characterisation, and exposure assessment for the genetically modified derivatives of *E. coli* BL21 (DE3), was provided in line with EFSA guidance (EFSA GMO Panel, 2011). The absence of bacteria from the Enterobacteriaceae family (ISO 21528-2) and residual bacterial DNA in the production strain (two antimicrobial resistance genes) and degradation strain (four antimicrobial resistance genes) confirmed that the genetically modified *E. coli* BL21 (DE3) strains were not present in the novel food.

Batch-to-batch analysis of the novel food confirmed the presence of very low levels of microbial endotoxins (highest value reported: 0.228 EU/mg) and residual proteins (< 100 µg/g) which were not considered to be a safety concern.

2.1.3. Compositional information and specification

The novel food specification is summarised in [Table 1](#).

Table 1. Specification of the novel food.

Parameter	Specification	Method of analysis
Lacto-N-tetraose	≥ 75% DM	HPAEC-PAD (internal validated method)
Lacto-N-triose II	≤ 5% DM	HPAEC-PAD (internal validated method) ***
D-lactose	≤ 5% DM	HPAEC-PAD (internal validated method) ***
<i>para</i> -lacto-N-hexaose	≤ 5% DM	HPAEC-PAD (internal validated method) ***
D-galactose and D-glucose *	≤ 5% DM	HPAEC-PAD (internal validated method) ***
Sum of other carbohydrates **	≤ 15% DM	Calculation
Water	≤ 9 %	Karl Fischer titration
Ash	≤ 1 %	ASU L 06.00-4
Protein content	≤ 100 µg/g	Nanoquant (modified Bradford)
Arsenic	≤ 0.2 mg/kg	ASU L 00.00-135: 2011-01 ICP-MS ****
Aflatoxin M1	≤ 0.025 µg/kg	DIN EN ISO 14501: 2008-01 IAC-HPLC-FD
Standard plate count	≤ 1,000 CFU/g	ISO 4833-2
Yeast and mould	≤ 100 CFU/g	ISO 21527-2: 2008-07
Enterobacteriaceae	≤ 10 CFU/g	ISO 21528-2: 2019-05
<i>Salmonella</i>	Absent in 25g	DIN EN ISO 6579-1: 2017-07
<i>Cronobacter spp.</i>	Absent in 10g	ISO/TS 22964: 2017-04
Endotoxins (EU/mg)	≤ 10 EU/mg	Ph. Eur. 2.6.14

ASU = Official collection of analysis methods according to § 64 of the German Food and Feed Code (LFGB); DM = dry matter; CFU = colony forming unit; DIN = German Institute for Standardisation e. V.; EN = European norm; EU/mg = endotoxin unit per milligram; IAC-HPLC-FD = Immunoaffinity chromatography – high-performance liquid chromatography – fluorescence detection; ICP-MS = Inductively coupled plasma – mass spectrometry; ISO = International Organisation for Standardisation; Ph. Eur. = European Pharmacopeia; TS = Technical specification

* D-galactose and D-glucose peaks on the HPAEC-PAD chromatograms overlap at 3.7 min retention time.

** Sum of other carbohydrates (% w/w DM) = 100 (% w/w DM) – LNT (% w/w DM) – Quantified carbohydrates (% w/w DM) – Ash (% w/w DM).

*** LOQ: D-lactose = 0.27% w/w DM; D-galactose and D-glucose = 0.14% w/w DM; Lacto-N-triose II = 0.54% w/w DM; *para*-lacto-N-hexaose = 0.81% w/w DM.

**** LOQ for arsenic = 0.05 mg/kg.

Batch-to-batch analysis confirmed that the novel food primarily consists of LNT (81.6 – 92.7 % w/w DM). Other saccharides are present and comply with the specification in [Table 1](#). D-lactose, D-glucose and D-galactose are recognised constituents of human breast milk. Lacto-N-triose II is found in human milk (Dabrowski et al., 1983; Hosomi & Takeya, 1988; Miwa et al.,

2010) as a metabolic intermediate of LNT biosynthesis and as one of the products of LNT hydrolysis (Kuhn et al., 1956). *Para*-lacto-N-hexaose was first isolated in human milk and described by Yamashita *et al.* (1977).

A stability study using an HMO mix (2'-FL – 47.1%; 3-FL – 16.0%; LNT – 23.7%; 3'-SL sodium salt – 4.1%; 6'-SL sodium salt – 4.0%; other carbohydrates – 5.1%) was conducted under real-time conditions (25°C and 60% relative humidity) and accelerated conditions (40°C and 75% relative humidity). The results from this study support the stability of the novel food for up to 12 months.

Information on the production process, composition, stability and the specification of the novel food does not raise safety concerns.

2.1.4. History of use

Human milk contains a family of structurally related oligosaccharides known as human milk oligosaccharides (HMOs). LNT belongs to the sub-fraction of neutral HMOs. The composition and concentration of HMOs vary between mothers' and over the course of lactation. A recent review of published studies reporting levels of LNT in mothers' milk (Erney et al., 2000) identified the mean of means and maximum mean for LNT as 0.76 g/L and 2.74 g/L, respectively.

Using these values and considering the average and high daily intake of breast milk (800 mL and 1,200 mL, respectively) for infants from 0 to 6 months (EFSA NDA Panel, 2013), the 95th percentile daily intake levels of LNT from human milk for a 6.7 kg body weight infant are 327 mg/kg BW/day (800 ml) and 491 mg/kg BW/day (1,200 ml).

LNT produced by fermentation using genetically modified *E. coli* BL21 (DE3), the novel food seeking authorisation, has no history of use as a novel food in GB but is currently authorised in the EU (assimilated Commission Implementing Regulation (EU) 2023/7) for the same intended food categories requested by the applicant.

LNT produced by fermentation using genetically modified *E. coli* K-12 DH1 (Glycom A/S, Denmark) is already authorised as a novel food in GB (assimilated Commission Implementing Regulation (EU) 2020/484) for use in foods for infants and young children, and food supplements.

In the USA, LNT produced by fermentation using genetically modified *E. coli* BL21 (DE3) is Generally Regarded as Safe (GRAS) as a food ingredient in foods for special groups and beverages (GRN 923 – FDA 2021; GRN 1017 – FDA 2022). In addition, LNT produced by fermentation using genetically modified *E. coli* K-12 DH1 is GRAS as a food ingredient (GRN 833 – FDA 2020).

2.1.5. Proposed use and intake

The target population is the general population. The intended food categories and maximum use levels are listed in [Table 2](#), which are the same as those listed in Commission Implementing Regulation (EU) 2017/2470.

Table 2. Food categories and use levels for LNT from the novel food.

Food category name	Maximum use level *
Infant formula, powder	1,456 mg/100g **
Infant formula, liquid	182 mg/100g
Follow-on formula, powder	1,456 mg/100g **
Follow-on formula, liquid	182 mg/100g
Cereals with an added high protein food which are reconstituted with water or other protein-free liquid	728 mg/100g **
Simple cereals which are reconstituted with milk or other appropriate nutritious liquids	1,274 mg/100g **
Cereals with an added high protein food reconstituted	182 mg/100g
Simple cereals for infants and children, reconstituted	182 mg/100g
Biscuits, rusks and cookies for children	182 mg/100g
Pasta for children (dry, to be cooked)	182 mg/100g
Ready-to-eat dairy-based meal for children	182 mg/100g
Special food for children's growth	182 mg/100g
Fruit and vegetable juices and nectars specific for infants and young children	182 mg/100g
Foods for infants and young children for special medical purposes as defined in Regulation (EU) No 609/2013	In accordance with the particular nutritional requirements of the persons for whom the products are intended
Foods for special medical purposes defined in Regulation (EU) No 609/2013 excluding foods for infants and young children	In accordance with the particular nutritional requirements of the persons for whom the products are intended
Food supplements *** for infants and young children	1.82 g/day
Food supplements *** for other children, adolescents and adults	4.6 g/day

* The proposed maximum use level for all food categories is 1.82 g/kg expressed as LNT which corresponds to a maximum use level of 2.10 g/kg when expressed as novel food (average 86.5% LNT based on batch-to-batch analysis).

** Relevant dilution factors (EFSA, 2018) have been used to calculate intake estimates.

*** Food Supplements as defined in the Food Supplements (England) Regulations 2003/1387, the Food Supplements (Wales) Regulations 2003/1719 and the Food Supplements (Scotland) Regulations 2003/278.

The anticipated intake for LNT in children up to the age of 16 weeks is estimated to be 473 mg/kg body weight/day, equivalent to 3.17 g/day for a 6.7 kg infant. This value was calculated from the use of LNT in infant formula (1.82 g/L) at a high consumption level of 260 ml/kg body

weight/day, as established by the EFSA Scientific Committee (EFSA Scientific Committee, 2017). This value does not exceed the estimated high-level daily intake for LNT of 491 mg/kg BW/day in breastfed infants.

An intake assessment using the summary statistics of consumption from the dietary surveys in the EFSA Comprehensive database was conducted by matching the proposed conditions of use with the FoodEx2 categories. The estimated mean and 95th percentile intakes of LNT from the proposed conditions of use for each sub-population are presented in [Table 3](#).

Table 3. Estimated daily intake of LNT on a body weight basis from the intended food uses only

Population group *	Mean intakes of LNT (mg/kg BW/day)	95 th percentile intakes of LNT (mg/kg BW/day)
Infants	42 – 185	157 – 403
Young children	3 – 61	25 – 153
Other children	0 – 3	0 – 20
Adolescents	0 – 1	0 – 5
Adults	0	0 – 2

BW = body weight

* Infants (≤ 11 months); young children (1 to < 3 years); other children (3 to < 10 years); adolescents (10 to < 18 years); adults (≥ 18 years) which includes pregnant and lactating women, elderly, and very elderly.

The estimated daily intake of LNT from the intended uses and use levels does not exceed the estimated high-level daily intake for LNT of 491 mg/kg BW/day in breastfed infants.

The estimated intake for LNT in food supplements on a body weight basis for all population groups at the proposed use levels is presented in [Table 4](#).

Table 4. Estimated daily intake of LNT from food supplements only on a body weight basis

Population group *	Proposed use level (g/day)	Mean body weight (kg) **	Estimated intake (mg/kg BW/day) ***
Infants	1.82	5	364
Young children	1.82	12	152
Other children	4.6	23.1	199
Young Adolescents	4.6	43.4	106
Older adolescents	4.6	61.3	75
Adults	4.6	70	66

BW = body weight

* Infants (≤ 11 months); young children (1 to < 3 years); other children (3 to < 10 years); adolescents (10 to < 18 years); adults (≥ 18 years) which includes pregnant and lactating women, elderly, and very elderly.

** EFSA Scientific Committee, 2012.

*** Calculation: [Proposed Use Level (g/day) / Mean Body Weights] x 1,000.

The use level for LNT in food supplements is 1.82 g/day for infants and young children, and 4.6 g/day for all other population sub-groups. These estimated daily intake levels do not exceed the estimated high-level daily intake for LNT of 491 mg/kg BW/day in breastfed infants.

Food supplements are not intended to be used if other foods with the novel food are consumed on the same day. For infants and young children, food supplements are not intended to be used if breast milk or other foods with added LNT are consumed on the same day.

LNT is already authorised in food categories other than those intended for the novel food in this safety assessment (beverages, flavoured and unflavoured fermented milk-based products, cereal bars). The combined daily intake of LNT from the authorised and proposed uses, for each population group from each EU dietary survey is shown in [Table 5](#).

Table 5. Estimated daily intake of LNT on a body weight basis from the authorised and intended food uses only at the maximum use levels.

Population group *	Mean intakes of LNT (mg/kg BW/day)	95 th percentile intakes of LNT (mg/kg BW/day)
Infants	66 – 235	172 – 563
Young children	44 – 126	124 – 351
Other children	14 – 73	37 – 182
Adolescents	5 – 19	20 – 47
Adults	8 – 13	29 – 31

BW = body weight

* Infants (≤ 11 months); young children (1 to < 3 years); other children (3 to < 10 years); adolescents (10 to < 18 years); adults (≥ 18 years) which includes pregnant and lactating women, elderly, and very elderly.

For most sub-populations, the estimated daily intake from authorised and intended food uses only does not exceed the estimated high-level daily intake for LNT of 491 mg/kg BW/day in breastfed infants. The only exception is the infant sub-population where highest high-level intake is 563 mg/kg BW/day, approximately 15% higher than the high estimate for LNT daily intake in breastfed infants. This is not considered a concern as the novel food is an alternative source of LNT for these product categories. It is noted that the calculation is conservative in nature as such individual consumers are unlikely to exceed the exposure levels from breast feeding.

There is no concern with respect to the exposure to undesirable substances from the consumption of novel food at the proposed uses.

2.1.6. Absorption, Distribution, Metabolism and Excretion (ADME)

Most human milk oligosaccharides are reported to undergo limited oral absorption intact. Human milk oligosaccharides do not undergo significant digestion in the upper gastrointestinal tract but can undergo fermentation in the colon. Human milk oligosaccharides are predominantly excreted unchanged in the faeces, with a small proportion excreted unchanged in the urine.

The absorption of LNT from consumption of the novel food is not expected to differ from the intake of human milk oligosaccharides following infant consumption of breast milk. Therefore, this was not expected to pose a safety concern for any age groups including infants.

The ADME of human milk oligosaccharides are well understood and the information does not indicate any further areas of concern.

2.1.7. Nutritional information

The novel food is primarily composed of the oligosaccharide, LNT, which is structurally identical to the naturally occurring counterpart in human breast milk. Consumption of the novel food at the proposed use levels is not expected to be nutritionally disadvantageous for consumers.

2.1.8. Toxicological information

Toxicological studies were performed with HMO mix which is composed of 2'-FL (47.1%), 3-FL (16.0%), LNT (23.7%), 3'-SL sodium salt (4.1%), 6'-SL sodium salt (4.0%) and other carbohydrates (5.1%) to support the safety assessment of the novel food.

2.1.8.1. Genotoxicity

In vitro genotoxicity testing of the HMO mix was conducted under Good Laboratory Practice (GLP) (OECD, 1998) conditions and according to the following OECD guidelines: *in vitro* bacterial reverse mutation test – OECD TG 471 (OECD, 1997) and *in vitro* mammalian cell micronucleus test – OECD TG 487 (OECD, 2016). The *in vitro* bacterial reverse mutation test (unpublished study, LPT No. 35908; Parschat et al., 2020) demonstrated that the HMO mix is non-mutagenic (up to 142 mg LNT/plate), in the absence or presence of metabolic activation. The *in vitro* mammalian cell micronucleus test (unpublished study, LPT No. 35909; Parschat et al., 2020) demonstrated that the HMO mix is non-clastogenic and non-aneugenic (up to 14 mg LNT/ml) in the absence or presence of metabolic activation. The results from these *in vitro* studies support the conclusion that the novel food is not genotoxic.

2.1.8.2. Sub-acute toxicity study

A seven-day pilot feeding study (unpublished study, LPT No. 35504; Parschat et al., 2020) was conducted under GLP conditions in two groups of five female rats fed either a standard diet or a standard diet plus 10% HMO mix. No deaths or differences in clinical signs, food consumption or body weight were reported. The 10% HMO mix dose was chosen for the repeated dose 90-day oral toxicity study.

2.1.8.3. Sub-chronic toxicity study

A repeated dose 90-day oral toxicity study in rodents (unpublished study, LPT No. 35907; Parschat et al., 2020) was conducted under GLP (OECD, 1998) according to OECD TG 408 guidelines (OECD, 2018). Each group consisted of 10 female and 10 male rats which were fed a standard diet or a standard plus 10% HMO mix *ad libitum*. There were no deaths, test item-related clinical abnormalities, or ocular changes. Episodes of decreased or increased food consumption were reported (male only). Statistically significant changes were observed in functional observation tests (male only). Variations in haematology (female only), clinical chemistry, urinalysis (female only), organ weights, and histopathology (male only) were observed.

The observed changes were of low magnitude and/or limited to one sex and were not considered to be biologically relevant. Therefore, the no observable adverse effect level (NOAEL) for the HMO mix was 5,670 mg/kg BW/day, which is equivalent to 1,340 mg/kg BW/day for LNT.

2.1.9. Allergenicity

The protein content in the novel food is low ($\leq 0.01\%$). This is supported by batch-to-batch analysis which consistently demonstrated that the protein levels were below the limit of quantification (0.001%).

The potential allergenicity of the introduced proteins expressed in *E. coli* BL21 (DE3) (Allergen Online tool, version 19 – University of Nebraska) was assessed using the 'higher than 35% identity in a sliding window of 80 amino acids' as the criterion (EFSA GMO Panel, 2010). None of the proteins was predicted to be an allergen.

Based on the information provided, the likelihood of allergenic reactions to the novel food is low.

3. Other regulators opinions and conclusions

The novel food is a purified white to ivory powder primarily composed of LNT. D-lactose, D-galactose and D-glucose, *para*-lacto-N-hexaose, lacto-N-triose and a small fraction of other related saccharides are also present. The novel food is manufactured by microbial fermentation using two strains of genetically modified *E. coli* BL21 (DE3), a production strain and an optional degradation strain.

The novel food is intended for the general population in foods for special medical purposes and food supplements, including those for vulnerable groups (pregnant and breastfeeding women, and the elderly). It is also intended to be used in food for infants and young children, including infant formula and follow-on formula.

LNT is a naturally occurring oligosaccharide present in human milk. The history of human exposure to LNT concerns breastfed infants; however, consumption of LNT is expected to be safe for other population groups.

LNT produced by genetically modified *E. coli* K-12 DH1, has previously been assessed by EFSA and received a positive opinion (EFSA NDA Panel, 2022).

Lacto-N-neotetraose (LNnT), a constitutional isomer of LNT, produced by fermentation using the same genetically modified *E. coli* BL21 (DE3) as the novel food, has also been assessed by EFSA with a positive outcome (EFSA NDA Panel, 2020).

The toxicology studies did not raise any safety concerns. No adverse effects were observed in the repeated dose 90-day oral toxicity study in rodents and the NOAEL for LNT was reported as 1.34 g LNT/kg BW/day. When this NOAEL is compared with the highest estimated exposure from authorised and intended uses in each population group, the margins of exposure range from 2.4 to 43. Given that the LNT in the novel food is equivalent to LNT found in human breast milk, these margins of exposure are acceptable with respect to the highest estimated daily intakes in the intended population.

The estimated high-level intakes of LNT by children up to 16 weeks of age consuming infant formula only, and from the intended food uses at their respective maximum use levels in all population groups, does not exceed the highest daily intake level of LNT in breastfed infants on a body weight basis. However, when the combined high-level intake of LNT from authorised and intended food uses is considered, the infant sub-population exceeds this value on a body weight basis by 15%.

The highest daily intake level of LNT in breastfed infants on a body weight basis is only exceeded in one out of twelve dietary surveys in the EFSA Food Consumption Database. In addition, the intake assessment assumes the novel food is added at the maximum use levels to all the intended food categories consumed by infants. On this basis, infants are not expected to exceed the high-level intake of LNT in breastfed infants on a body weight basis.

The maximum daily intake of LNT in food supplements is 1.82 g/day for infants and young children, and 4.6 g/day for the general population. These levels do not exceed the highest intake level of LNT in breastfed infants on a body weight basis. Food supplements are not intended to be used if other foods with added LNT (as well as human milk for infants and young children) are consumed on the same day.

Consumption of the novel food under the proposed conditions of use is not expected to be a safety concern because of the limited absorption of human milk oligosaccharides, the absence of adverse effects reported in the toxicology studies, and the fact that breastfed infants are naturally exposed to these substances without adverse effects at the intended use level.

4. Uncertainties and limitations

The FSA and FSS noted that no specific uncertainties were flagged in the assessment.

The FSA and FSS could not have completed the safety assessment of the novel food under the proposed conditions of use without the following data claimed as proprietary by the applicant:

- i. identity of the novel food as confirmed by MS, NMR spectroscopy and HPAEC-PAD
- ii. toxicological information, including *in vitro* genotoxicity studies, subacute and sub-chronic toxicity studies
- iii. description of the genetically modified LNT and LNnT production and optional degradation strains, qPCR detection system and method validation reports for the LNT production and optional degradation strains, certificates of deposition of the LNT production and optional degradation strains, and genome sequence of the parental strain *E. coli* BL21 (DE3)
- iv. method validation reports for the determination of LNT and carbohydrate by-products in the novel food using HPAEC-PAD.

5. FSA-FSS conclusion for GB safety assessment

The application has been evaluated in line with 'Guidance on the preparation and presentation of an application for authorisation of a novel food in the context of assimilated Regulation (EU) 2015/2283 (EFSA NDA Panel, 2016), and assimilated Commission Implementing Regulation (EU) 2017/2469, for purposes of the GB safety assessment.

The conclusions of the EFSA opinion (EFSA NDA Panel, 2022), which have been reviewed in detail by the FSA and FSS for the purposes of the GB safety assessment, are considered appropriate and consistent within the uncertainties and limitations identified by EFSA.

6. Outcome of the assessment

The FSA and FSS has reviewed the applicant's dossier, supporting documentation, and most notably the EFSA opinion (EFSA NDA Panel, 2022), and consider that there is sufficient evidence to conclude the safety assessment of lacto-N-tetraose (LNT) without obtaining further information or conducting a further risk assessment.

The FSA and FSS conclude that LNT is safe under the proposed conditions of use. The anticipated intake levels and the proposed use in food and food supplements is not considered to be nutritionally disadvantageous.

In making this assessment, the FSA and FSS were able to rely on sufficient scientific evidence to make a conclusion on safety with no further questions to the applicant, and therefore no further risk assessment activities are necessary.

Sufficient evidence was available in the literature to give the FSA and FSS confidence about the safety of this novel food, for example, where other national food safety authorities had positively assessed the application using the same risk assessment guidance and core legal requirements which apply in GB.

Applicants provided sufficient relevant information as requested by the FSA and FSS.

The FSA and FSS review did not find any issues of divergence from the EFSA guidance (EFSA NDA Panel, 2021) or mutual approaches or new scientific issues for consideration.

There were no other specific issues that would require an assessment for the UK or the nations of the UK.

Abbreviations

2'-FL	2'-fucosyllactose
3-FL	3-fucosyllactose
3'-SL	3'-siallylactose
6'-SL	6'-siallylactose
ACNFP	Advisory Committee on Novel Foods and Processes
ADME	Absorption, Distribution, Metabolism and Excretion
ASU	Official collection of analysis methods according to § 64 of the German Food and Feed Code (LFGB)
BW	Body weight
CAS	Chemical Abstracts Service
CFU	Colony Forming Unit
COSY	Correlated spectroscopy
DIN	German Institute for Standardisation e. V
DM	Dry matter
EFSA	European Food Safety Agency
EN	European norm
EU	European Union
EU/mg	Endotoxin unit per milligram
FDA	Food and Drug Administration
FSA	Food Standards Agency
FSS	Food Standards Scotland
GB	Great Britain
GLP	Good Laboratory Practice
GMM	Genetically modified organism
GRAS	Generally regarded as safe
GRN	GRAS Notification
HMBC	heteronuclear multiple bond correlation
HMO	Human milk oligosaccharide
HPAEC	High-Performance Anion-Exchange chromatography
HPLC-MS	High Performance Liquid Chromatography – Mass Spectrometry
HSQC	heteronuclear single quantum correlation
IAC-HPLC-FD	Immunoaffinity chromatography – high-performance liquid chromatography – fluorescence detection
ICP-MS	Inductively coupled plasma – mass spectrometry
ISO	International Organisation for Standardisation
LNT	Lacto-N-tetraose
LOD	Limit of detection
LOQ	Limit of quantification
NMR	Nuclear magnetic resonance
NOAEL	No Observed Adverse Effect Level
OECD	Organisation for Economic Cooperation and Development
PAD	Pulsed Amperometric Detection

Ph. Eur.	European Pharmacopeia
TS	Technical specification



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