REGULATED PRODUCTS SAFETY ASSESSMENT

Assessment of 3-Fucosyllactose (3-FL) as a Novel Food for Use in Food and Food Supplements (RP2106)

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FSA Research and Evidence

Chr. Hansen, Denmark ("the applicant") submitted a novel food application for the authorisation of 3-fucosyllactose (3-FL) 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, 3-FL, 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 3-FL 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 May 2022. The Food Standards Agency (FSA) and Food Standards Scotland (FSS) have reviewed the information available, including the EFSA opinion, and confirmed that 3-FL was 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 2283/2015 on novel foods, the application RP2106 for the use of 3-fucosyllactose (3-FL) 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. When GB left the

EU, it retained the same regulations for food and feed regulated products; the FSA and FSS also adopted equivalent technical guidance and quality assurance processes (EFSA NDA, 2016) 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 provide a summary assessment of the evidence of safety presented in this report.

Specifically, in reviewing the risk assessment that EFSA have recently completed, the reviewers have verified that the standard approach taken, when compared to the relevant guidance applied in GB, has been followed and the conclusions made are consistent with the data summarised in the opinion. 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 3-FL as a novel food produced by fermentation using genetically modified *E. coli* BL21 (DE3).

The first novel food application for 3-FL, which was submitted by DuPont Nutrition and Biosciences ApS (Denmark), is produced by fermentation using genetically modified *E. coli* K-12 MG1655. A positive EFSA opinion was published in June 2021 (EFSA NDA Panel, 2021). This novel food is currently authorised in EU (assimilated Commission Implementing Regulation (EU) 2021/2029) for use in dairy products and analogues, bakery wares, foods for special groups, beverages and food supplements.

3-FL produced by fermentation using genetically modified *E. coli* BL21 (DE3) (Jennewein Biotechnologie GmbH, Germany – wholly owned by Chr. Hansen A/S, Denmark) is Generally Regarded as Safe (GRAS) (FDA, 2021a) for use as an ingredient in infant formula only. A second application in August 2023 for 3-FL (Chr. Hansen A/S, Denmark), using the same genetically modified organism, is GRAS (FDA, 2023) as a food ingredient in foods for special groups and beverages.

In April 2022, the safety of 3-FL 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 ≥ 90% 3-FL by dry weight. Other saccharides are present in smaller quantities: D-lactose (≤ 5% w/w dry weight), L-fucose (≤ 3% w/w dry weight), D-galactose (≤ 3% w/w dry weight), and D-glucose (≤ 3% w/w dry weight), and a small fraction of other related saccharides (sum of other carbohydrates ≤ 5% w/w dry weight).

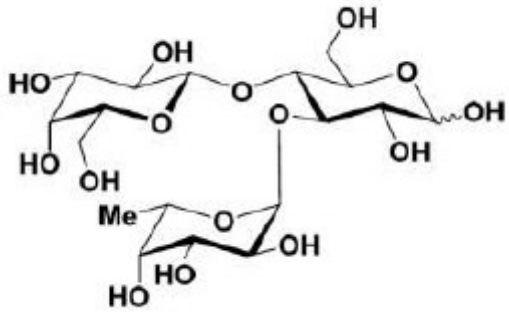

Figure 1. The structural formula of 3-fucosyllactose (3-FL).

3-FL is a trisaccharide consisting of D-galactose, L-fucose and D-glucose (see Figure 1), where the L-fucose is linked to the glucose moiety in D-lactose via an α-(1-3) bond.

3-FL is a constitutional isomer of 2'-fucosyllactose (2'-FL). Both isomers contain the same monosaccharides but there is an α-(1-2') bond between L-fucose and the D-galactose moiety of D-lactose in 2'-FL.

3-FL is characterised by the following information:

- IUPAC name: (*2S,3S,4R,5S,6S*)-2-[(*3R,4R,5R,6R*)-2,3-dihydroxy-6-(hydroxymethyl)-5-[(*2S,3R,4S,5R,6R*)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxyoxan-4-yl]oxy-6-methyloxane-3,4,5-triol
- CAS number: 41312-47-4
- Molecular formula: $C_{18}H_{32}O_{15}$
- Molecular mass: 488.44 g/mol

The molecular structure of the 3-FL in the novel food was determined by high-performance liquid chromatography – electrospray ionisation – tandem mass spectrometry (HPLC-ESI-MS/MS). The collision induced decay (CID) fragmentation patterns from the 3-FL 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 3-FL in the novel food, is equivalent to 3-FL found in human breast milk was provided by comparative nuclear magnetic resonance (NMR) spectroscopy: mono-dimensional (1D) nuclear magnetic resonance (NMR) spectroscopy including ^{1}H-, ^{13}C- and ^{13}C-DEPT-135 (distortionless enhancement by polarisation transfer) spectra, and two-dimensional (2D) NMR spectroscopy, including ^{1}H-^{1}H COSY (correlated spectroscopy), ^{1}H-^{13}C HSQC (heteronuclear single quantum correlation) and ^{1}H-^{13}C HMBC (heteronuclear multiple-bond correlation) spectra, by comparison with a commercially available 3-FL standard.

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 3-FL using a genetically modified microorganism (GMM) derived from *E. coli* BL21 (DE3). 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 ≥ 90% 3-FL 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 (five 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: 0.023 EU/mg) and residual proteins (< 0.001 %) 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
3-fucosyllactose	≥ 90% DM	HPAEC-PAD (internal validated method)
D-lactose	≤ 5% DM	HPAEC-PAD (internal validated method) ***
L-fucose	≤ 3% DM	HPAEC-PAD (internal validated method) ***
D-galactose*	≤ 3% DM	HPAEC-PAD (internal validated method) ***
D-glucose*	≤ 3% DM	HPAEC-PAD (internal validated method) ***
Sum of other carbohydrates**	≤ 5% DM	Calculation
Water	≤ 9 %	Karl Fischer titration
Ash	≤ 1 %	ASU L 06.00-4
Protein content	≤ 0.01 %	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
Salmonella	Absent in 25g	DIN EN ISO 6579-1: 2017-07
Cronobacter spp.	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) – 3-FL (% w/w DM) – Quantified carbohydrates (% w/w DM) – Ash (% w/w DM).

*** LOQ: D-Lactose = 0.14% w/w DM; D-Galactose = 0.14% w/w DM; D-Glucose = 0.14% w/w DM; L-Fucose = 0.28% w/w DM.

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

Batch-to-batch analysis confirmed that the novel food primarily consists of 3-FL (94.1 – 97.4 % 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. L-Fucose is also found in human milk (Smilowitz et al., 2013) at concentrations ranging from 20 to 30 mg/L (Choi et al., 2015).

A stability study using an HMO mix (2'-FL – 47.7%; 3-FL – 15.1%; LNT – 24.7%; 3'-SL sodium salt – 4.3%; 6'-SL sodium salt – 5.6%; other carbohydrates – 5.7%) was conducted under real-time conditions (25^{O}C and 60% relative humidity) and accelerated conditions (40^{O}C and 75% relative humidity). The results from this study support the stability of the novel food for up to 24 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). 3-FL 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 3-FL in mothers' milk (Soyyılmaz et al., 2021) identified the mean of means and maximum mean for 3-FL as 0.92 g/L and 2.57 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 3-FL from human milk for a 6.7 kg body weight infant are 307 mg/kg BW/day (800 ml) and 460 mg/kg BW/day (1,200 ml).

3-FL 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) 2023/52) for the same intended food categories requested by the applicant.

3-FL produced by fermentation using genetically modified *E. coli* K-12 MG1655 (DuPont Nutrition and Biosciences ApS, Denmark) is already authorised as a novel food in EU (assimilated Commission Implementing Regulation (EU) 2021/2029) for use in beverages, dairy products and analogues, bakery wares, foods for special groups, and food supplements.

In the USA, 3-FL produced by fermentation using genetically modified *E. coli* BL21 (DE3) is Generally Regarded as Safe (GRAS) as a food ingredient in foods for beverages, dairy products and analogues, bakery wares and

foods for special groups (FDA, 2021a; FDA, 2023). In addition, 3-FL produced by fermentation using genetically modified *E. coli* K-12 MG1655 (FDA, 2021b) and genetically modified *E. coli* K-12 DH1 (FDA, 2022) is GRAS as a food ingredient.

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 assimilated Commission Implementing Regulation (EU) 2023/52.

Table 2. Food categories and use levels for 3-FL from the novel food.

Food category name	Maximum use level
Infant formula as defined under assimilated Regulation (EU) No 609/2013, powder	720 mg/100g *
Infant formula as defined under assimilated Regulation (EU) No 609/2013, liquid	90 mg/100g
Follow-on formula as defined under assimilated Regulation (EU) No 609/2013, powder	960 mg/100g *
Follow-on formula as defined under assimilated Regulation (EU) No 609/2013, liquid	120 mg/100g
Cereals with an added high protein food which are reconstituted with water or other protein-free liquid	480 mg/100g *
Simple cereals which are reconstituted with milk or other appropriate nutritious liquids	840 mg/100g *
Cereals with an added high protein food reconstituted	120 mg/100g
Simple cereals for infants and children, reconstituted	120 mg/100g
Biscuits, rusks and cookies for children	120 mg/100g
Pasta for children (dry, to be cooked)	120 mg/100g
Ready-to-eat dairy-based meal for children	120 mg/100g
Special food for children's growth	120 mg/100g
Fruit and vegetable juices and nectars specific for infants and young children	120 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	1.2 g/day
Food supplements *** for other children, adolescents and adults	3.0 g/day

* Relevant dilution factors 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, for infants and children (under 3 years).

*** 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, excluding food supplements for infants and young children (under 3 years).

The anticipated intake for 3-FL in children up to the age of 16 weeks is estimated to be 234 mg/kg body weight/day, equivalent to 1.57 g/day for a 6.7 kg infant. This value was calculated from the use of 3-FL in infant formula (0.9 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 3-FL of 460 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 3-FL from the proposed conditions of use for each sub-population are presented in Table 3.

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

Population group *	Mean intakes of 3-FL (mg/kg BW/day)	95th percentile intakes of 3-FL (mg/kg BW/day)
Infants	22 – 100	80 – 199
Young children	1 – 40	13 – 96
Other children	0 – 2	0 – 13
Adolescents	0 – 1	0 – 3
Adults	0	0 – 1

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 3-FL from the intended uses and use levels does not exceed the estimated high-level daily intake for 3-FL of 460 mg/kg BW/day in breastfed infants.

The estimated intake for 3-FL 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 3-FL 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	1.2	5	240
Young children	1.2	12	100
Other children	3.0	23.1	130
Young Adolescents	3.0	43.4	69
Older adolescents	3.0	61.3	49

Population group *	Proposed use level (g/day)	Mean body weight (kg) **	Estimated intake (mg/kg BW/day) ***
Adults	3.0	70	43

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 SC 2012.
*** Calculation: [Proposed Use Level (g/day) / Mean Body Weights] x 1,000.

The use level for 3-FL in food supplements is 1.2 g/day for infants and young children, and 3.0 g/day for all other population sub-groups. These estimated daily intake levels do not exceed the estimated high-level daily intake for 3-FL of 460 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 3-FL are consumed on the same day.

3-FL 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 3-FL 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 3-FL on a body weight basis from the authorised and intended food uses only at the maximum use levels.

Population group *	Mean intakes of 3-FL (mg/kg BW/day)	95th percentile intakes of 3-FL (mg/kg BW/day)
Infants	35 – 149	90 – 366
Young children	31 – 104	82 – 254
Other children	12 – 49	31 – 119
Adolescents	4 – 16	16 – 38
Adults	6 – 10	20 – 24

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 all sub-populations, the estimated daily intake from the authorised and intended food uses only does not exceed the estimated high-level daily intake for 3-FL of 460 mg/kg BW/day in breastfed infants.

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 3-FL 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, 3-FL, 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) conditions and according to the following OECD guidelines: *in vitro* bacterial reverse mutation test (OECD TG 471) and *in vitro* mammalian cell micronucleus test (OECD TG 487). 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 96 mg 3-FL/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 10 mg 3-FL/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 conditions according to OECD TG 408 guidelines. 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.

These 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 910 mg/kg BW/day for 3-FL.

2.1.9. Allergenicity

The protein content in the novel food is low (≤ 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 3-FL. D-lactose, D-galactose, D-glucose, L-fucose, and a small fraction of other related saccharides are also present. The novel food is manufactured by microbial fermentation using genetically modified *E. coli* BL21 (DE3).

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.

3-FL is a naturally occurring oligosaccharide present in human milk. The history of human exposure to 3-FL concerns breastfed infants; however, consumption of 3-FL is expected to be safe for other population groups.

3-FL produced by genetically modified *E. coli* BL21 (DE3), has previously been assessed by EFSA and received a positive opinion (EFSA NDA Panel, 2022).

2'-fucosyllactose (2'-FL), a constitutional isomer of 3-FL, produced by fermentation using the same genetically modified *E. coli* BL21 (DE3) is authorised as a novel food (Commission Implementing Regulation (EU) 2017/2201).

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 3-FL was reported as 0.91 g 3-FL/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 2.5 to 38. Given that the 3-FL in the novel food is equivalent to 3-FL 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 3-FL by children up to 16 weeks of age consuming infant formula only does not exceed the highest daily intake level of 3-FL in breastfed infants on a body weight basis. The anticipated daily intakes for 3-FL from the intended uses and the authorised plus intended uses at their respective maximum use levels in all populations are 199 mg/kg BW/day and 366 mg/kg BW/day, respectively. These values are below the highest daily intake level of 3-FL in breastfed infants on a body weight basis.

The maximum daily intake of 3-FL in food supplements is 1.2 g/day for infants and young children, and 3.0 g/day for the general population. These levels do not exceed the highest intake level of 3-FL in breastfed infants on a body weight basis. Food supplements are not intended to be used if other foods with added 3-FL (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 production strain, qPCR detection system and method validation reports for the 3-FL production and optional degradation strains, certificates of deposition of the 3-FL production strain, and genome sequence of the parental strain *E. coli* BL21 (DE3)
iv. method validation reports for the determination of 3-FL 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 application, supporting documentation, and other regulators risk assessments, most notably the EFSA risk assessment opinion (EFSA NDA Panel, 2022) and consider sufficient evidence has been demonstrated to conclude without further questions or risk assessment.

The FSA and FSS conclude that 3-fucosyllactose (3-FL) was safe under the proposed conditions of use. The anticipated intake levels and the proposed use in food and food supplements was 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 FSA/ FSS.

The FSA and FSS review did not find any issues of divergence from the EFSA guidance (EFSA NDA Panel, 2016) 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
OECD	Organisation for Economic Cooperation and Development
PAD	Pulsed Amperometric Detection
Ph. Eur.	European Pharmacopeia
TS	Technical specification

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