REGULATED PRODUCTS SAFETY ASSESSMENT

Assessment on 2'-Fucosyllactose (2'-FL) Derived From Genetically Modified *Corynebacterium Glutamicum* APC199 as a Novel Food for Use in Food and Food Supplements (RP2119)

Food Standards Agency[1], Food Standards Scotland[2]

[1] Regulated Products Risk Assessment, Food Standards Agency, UK, [2] Risk Assessment, Food Standards Scotland, UK

Keywords: Regulated Products, Safety assessment, Novel foods, Genetically Modified Organisms

https://doi.org/10.46756/001c.126854

FSA Research and Evidence

Advanced Protein Technologies Corp. ("the applicant") submitted a novel food application for the authorisation of 2'-Fucosyllactose (2'-FL) as a novel food to each nation of Great Britain in August 2023.

The novel food is intended to be used as a source of human identical milk oligosaccharide, 2'-FL, and is manufactured by microbial fermentation using a genetically modified strain of *Corynebacterium glutamicum* APC199. This new application is seeking to use the novel food produced by a new production organism within food, infant and follow-on formula, and food supplements.

This novel food had its application for authorisation assessed by the European Food Safety Authority (EFSA) which was published in December 2022. The Food Standards Agency (FSA) and Food Standards Scotland (FSS) have reviewed the information available, including the EFSA opinion, and confirmed that 2'-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.

This is a joint FSA and FSS publication.

1. Introduction

In accordance with assimilated Regulation 2283/2015 on novel foods, the application RP2119 for the use of 2'-Fucosyllactose derived from genetically modified *Corynebacterium glutamicum* APC199 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, Advanced Protein Technologies Corp., is seeking authorisation for 2'-FL as novel food derived from genetically modified *Corynebacterium glutamicum* APC199.

2'-FL, a trisaccharide consisting of L-fucose, D-galactose, and D-glucose produced by either chemical synthesis or microbial fermentation using derivate strains of *Escherichia coli* K-12 (DH1) or *Escherichia coli* BL21 (DE3) is already authorised to be used in a number of food products including food supplements (FS) as defined in Directive 2002/46/EC. 2'-FL is included in the Union list of authorised Novel Foods (Commission Implementing Regulation (EU) 2017/2470) and has since been authorised

to be produced in a number of production organisms. However, this novel food is being produced by another production organism not yet authorised and therefore assessment under the novel food regulations is required.

A 2'-FL/difucosyllactose (DFL), a mixture produced by fermentation with a genetically modified strain of *E. coli* K-12 and 3-fucosyllactose (3-FL), a constitutional isomer of 2'-FL produced by fermentation with a derivative strain of *E. coli* K-12 (MG1655), are also included in the Union list of authorised Novel Foods (NFs). The extension of use in FS for infants of 2'-FL and 2'-FL/DFL mixture, both produced with derivative strains of *E. coli* K-12 DH1, and the safety of 3-FL produced with a derivative strain of *E. coli* BL21 (DE3), have recently been assessed by EFSA with positive outcomes (EFSA NDA Panel, 2022 a,b,c).

Several scientific opinions have been adopted by the EFSA NDA Panel since 2015, on the safety of human-identical milk oligosaccharides (HiMOs) as NFs pursuant to Regulation (EC) No 258/97 or Regulation (EU) 2015/2283.

The primary difference in the specifications for 2'-FL from different microbial sources is limited to the carbohydrate components, specifically the levels of difucosyllactose (DFL), lactose, fucose, 3-fucosyllactose, fucosylgalactose, 2'-fucosyl-D-lactulose, glucose, and galactose. These differences are not considered to be a safety concern, as they relate to innocuous and structurally related carbohydrate components.

APTech's 2'-FL derived from genetically modified *Corynebacterium glutamicum* APC199 has a Generally Recognized as Safe (GRAS) notice re-submitted in April 2020 after data gaps were raised (GRN 932). The Food and Drug Agency issued a "no questions" letter in February of 2021 (U.S. FDA, 2021). A number of other 2'-FL ingredients produced by chemical synthesis or microbial fermentation have made a GRAS notification for use in infant formula (GRNs 546, 571, 650), as well as other food products such as baby foods (GRN 650 – U.S. 2015a, b, 2016).

In Israel, 2'-FL obtained from microbial fermentation of a strain derived from *E. coli* BL21 (DE3) is authorised as a novel food for use in milk-based infant formulas (ages 0 to 6 months) and follow-on formulas (ages 6 to 12 months) of the ready-to-feed product (Israel MOH, 2019). There are no authorisations for 2'FL produced using the microorganism in this application.

In December 2022, the safety of 2'-FL derived from *Corynebacterium glutamicum* APC199 as a novel food manufactured by Advanced Protein Technologies Corp. ("APTech") was assessed by EFSA and received a positive opinion (EFSA NDA Panel, 2022). This opinion has been reviewed by FSA/FSS risk assessors to inform the assessment of the novel food.

2.1. Methodology applied in the EFSA Opinion

The EFSA Assessment was in accordance with the procedure as lined out in the EFSA scientific opinion 'Guidance on the preparation and presentation of an application for authorisation of a novel food in the context of Regulation EU (2015/2283)' (EFSA NDA Panel, 2016) and assimilated Commission Implementing Regulation (EU) 2017/2469.

2.1.1. Identity of the novel food

The Novel food is a powdered mixture of 2′-FL (97%) with trace levels of other carbohydrates such as lactose, DFL, glucose, and galactose. The Novel food is demonstrated by 1H- and 13C-nuclear magnetic resonance (NMR) spectroscopy to be chemically and structurally identical to 2′-FL that is produced synthetically and is naturally present in human breast milk. 2′-FL is a trisaccharide consisting of L-fucose linked via an a-(1-2′) bond to the D-galactose moiety of D-lactose and is characterised by the following information:

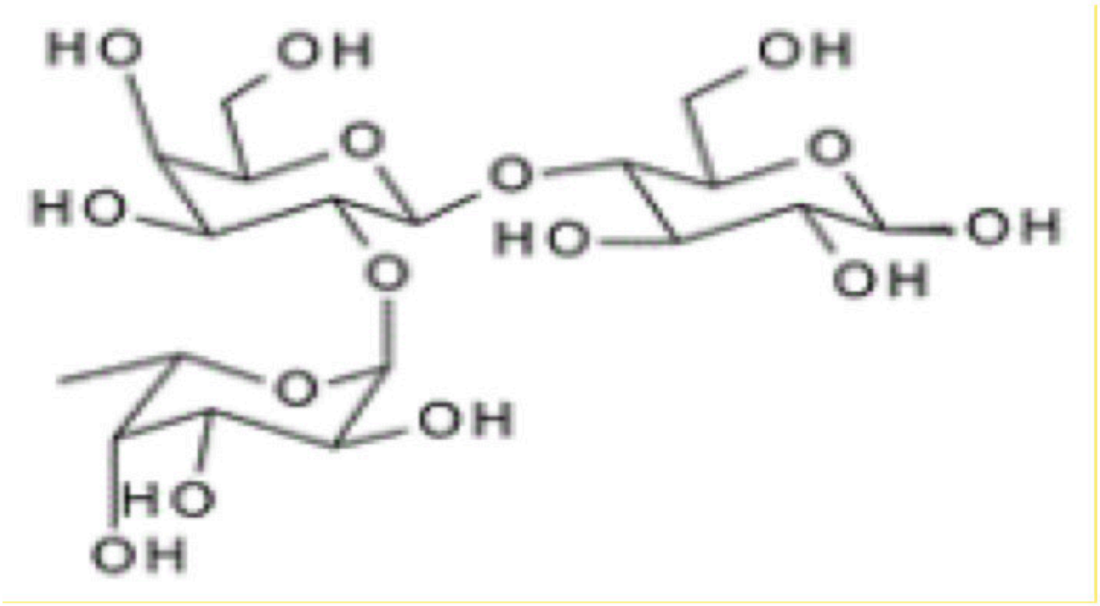

Figure 1. The structural formula of 2′-FL

IUPAC name: a-L-Fucopyranosyl-(1→2)-b- D-galactopyranosyl-(1→4)-D -glucopyranose

Synonyms: 2′-Fucosyl-D -lactose; 2′-O-fucosyllactose; 2′-FL

CAS number: 41263-94-9

Molecular formula: $C_{18}\ H_{32}\ O_{15}$

The identity of 2′-FL was also confirmed by high-performance anion-exchange chromatography–pulsed amperometric detection (HPAEC–PAD) by comparison with a high purity standard. The molecular structure of 2′-

FL was determined by liquid chromatography–tandem mass spectrometry (LC–MS/MS) based on the retention time, accurate mass and fragmentation pattern, which allowed to differentiate between 2′-FL characterised by a 1″-2′ bond and 3′-FL characterised by a 1″-3′ bond as well as being by comparison with a high purity standard.

2.1.2. Production Process

The Novel Food is produced in line with Good Manufacturing Practice (GMP) and Hazard Analysis Critical Control Points (HACCP) principles, in a facility that is FSSC (Food Safety System Certification) 22000 certified.

2′-FL is produced by fermentation using a genetically modified strain derived from the host strain *C. glutamicum* ATCC 13032. The production strain *C. glutamicum* APC199 has been modified to effectively synthesise 2′-FL. Glucose and lactose are used as carbon source and substrate, respectively, by *C. glutamicum* APC199 to produce 2′-FL, which is released into the fermentation media. At the end of the fermentation process, the bacterial biomass is heat inactivated and removed by microfiltration. The isolation, purification and concentration of the product involve a series of filtration steps and drying to obtain purified 2′-FL in powder form.

The production strain *C. glutamicum* APC199 is a genetically modified derivative of the host strain *C. glutamicum* ATCC 13032. *C. glutamicum* has been recommended for the qualified presumption (QPS) status but only for production purposes (EFSA BIOHAZ Panel, 2022; EFSA Scientific Committee, 2007) suggesting the absence of viable cells of the production microorganism in the final product should be a consideration in establishing safety.

The production organism is considered a Category 1 under EFSA's guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use (EFSA GMO Panel, 2010). This refers to the organism being used for the production of another substance. The focus for managing risk is to ensure the organism and any recombinant DNA are not present in the final food.

Four antimicrobial resistance genes either introduced during the genetic modification or identified in the whole genome sequence (WGS) analysis of the production strain, had been previously identified in the bioinformatic analysis performed in accordance with the EFSA statement on WGS analysis (EFSA, 2021).

No residual DNA from the production strain was detected in the 2′FL by quantitative polymerase chain reaction (qPCR) amplification of 10 target genes. In accordance with the EFSA Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA

FEEDAP Panel, 2018) the absence of both DNA and viable cells from the production strain provides reassurance that there are not risks from contamination of the novel food from the production organism.

2.1.3. Compositional information and Specification

Batch-to-batch analyses confirmed that the novel food consists of 2'-FL (>97% w/w DM) as the main component. The remaining constituents include D-lactose (< 0.14% w/w DM), L-fucose (< 0.07% w/w DM), 3-FL (< 0.32% w/w DM), DFL (< 0.28% w/w DM), D -glucose (< 0.28% w/w DM) and D-galactose 0.18% w/w DM).

The novel food is a white to off-white/ivory powder. The solubility in water was measured in five batches of the novel food according to the Organisation for Economic Cooperation and Development (OECD) test guideline (TG) 105 (OECD, 1995), resulting in an average value of 716.6 g/L at 20°C.

In order to confirm that the manufacturing process is reproducible and adequate to produce on a commercial scale a product with certain characteristics, the applicant provided analytical information for five batches of 2'-FL. Information was provided on the accreditation of the laboratories that conducted the analyses was presented in the application. Specification of the novel food is provided in Table 1.

Table 1. Specification of the novel food

Description: 2'-Fucosyllactose (2'-FL) is a white to off-white/ivory powder produced by microbial fermentation and further isolated and purified. **Source**: A genetically modified strain (APC199) of *Corynebacterium glutamicum* ATCC 13032	
Parameter	**Specification**
Composition	
2'-FL (% w/w DM)	≥ 94
D -Lactose (% w/w DM)	≤ 3
L-Fucose (% w/w DM)	≤ 3
3-FL (% w/w DM)	≤ 3
DFL (% w/w DM)	≤ 2
D -Glucose (% w/w DM)	≤ 3
D -Galactose (% w/w DM)	≤ 3
Water (%)	≤ 9.0
Ash (%)	≤ 0.5
Protein (%)	≤ 0.005
Contaminants	
Arsenic (mg/kg)	≤ 0.03
Aflatoxin M1 (µg/kg)	≤ 0.025
Ethanol (mg/kg)	≤ 1,000
Microbial parameters	

Description: 2'-Fucosyllactose (2'-FL) is a white to off-white/ivory powder produced by microbial fermentation and further isolated and purified. **Source**: A genetically modified strain (APC199) of *Corynebacterium glutamicum* ATCC 13032	
Total plate count (CFU/g)	≤ 500
Yeast and mould (CFU/g	≤ 100
Enterobacteriaceae (in 10 g)	ND
Salmonella (in 25 g)	ND
Cronobacter spp. (in 10 g)	ND
Endotoxins (EU/g)	≤ 100

2'-FL: 2'-Fucosyllactose; 3-FL: 3-Fucosyllactose; CFU: Colony forming unit; DFL: Difucosyllactose; DM: Dry matter; EU: Endotoxin unit; ND: Not detected.

Shelf-life stability studies were carried out on five batches of the novel food under normal storage conditions (25^{o}C, 60% relative humidity) and under accelerated conditions (40^{o}C, 75% relative humidity). The results from these studies 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

2'FL is a human milk Oligosaccharide found naturally in breast milk. 2'-FL is the most represented oligosaccharide in human milk. The level of 2'FL in breastmilk is considered safe for infants and young children.

There is no history of use for 2'-FL produced from genetically modified *Corynebacterium glutamicum* APC199 in GB but currently has a positive opinion (EFSA NDA Panel, 2022) for the same intended food categories (assimilated Commission Implementing Regulation (EU) 2023/950).

2'-FL is already included in the GB list of NFs when manufactured by chemical synthesis or fermentation with derivative strains of *E. coli* K-12 DH1 or *E. coli* BL21 (DE3). It is authorised to be added to a variety of food categories including foods for special groups such as infant formula and follow-on formula, and food supplements (FS), excluding FS for infants (intended for individuals above 1 year of age).

2.1.5. Proposed Use and Intake

The applicant does not intend to amend the uses and use levels already authorised for 2'-FL manufactured by chemical synthesis or fermentation with derivative strains *of E. coli* K-12 DH1 or *E. coli* BL21 (DE3). The intended food categories and maximum use levels therefore remain the same as those listed in assimilated Commission Implementing Regulation (EU) 2023/950 (Table 2).

Table 2. Food Categories and Use Levels for 2′-FL from the novel food

Food Category Name	Maximum Use Level	Additional specific labelling requirements
Unflavoured pasteurised and sterilised (including UHT) milk-based products	1.2 g/l	1. The designation of the novel food on the labelling of the foodstuffs containing it shall be "2′-fucosyllactose". 2. The labelling of food supplements containing 2′-fucosyllactose shall bear a statement that the supplements should not be used if other foods with added 2′-fucosyllactose are consumed the same day. 3. The labelling of food supplements containing 2′-fucosyllactose intended for young children shall bear a statement that the supplements should not be used if breast milk or other foods with added 2′-fucosyllactose are consumed the same day.'
Unflavoured fermented milk-based products	1.2 g/l for beverages 19.2 g/kg for products other than beverages	
Flavoured fermented milk-based products including heat-treated products	1.2 g/l for beverages 19.2 g/kg for products other than beverages	
Dairy analogues, including beverage whiteners	1.2 g/l for beverages 12 g/kg for products other than beverages 400 g/kg for whitener	
Cereal bars	12 g/kg	
Table-top sweeteners	200 g/kg	
Infant formula as defined in Regulation (EU) No 609/2013[a]	1.2 g/l in the final product ready for use, marketed as such or reconstituted as instructed by the manufacturer	
Follow-on formula as defined in Regulation (EU) No 609/2013[a]	1.2 g/l in the final product ready for use, marketed as such or reconstituted as instructed by the manufacturer	
Processed cereal-based foods and baby foods for infants and young children as defined in Regulation (EU) No 609/2013[a]	12 g/kg for products other than beverages 1.2 g/l for liquid food ready for use, marketed as such or reconstituted as instructed by the manufacturer	
Milk based drinks and similar products intended for young children (1-3 years)	1.2 g/l for milk-based drinks and similar products in the final product ready for use, marketed as such or reconstituted as instructed by the manufacturer	
Foods for special medical purposes as defined in Regulation (EU) No 609/2013[a]	In accordance with the particular nutritional requirements of the persons for whom the products are	

Food Category Name	Maximum Use Level	Additional specific labelling requirements
	intended	
Total diet replacement for weight control as defined in Regulation (EU) No 609/2013[a]	4.8 g/l for drinks 40 g/kg for bars	
Bread and pasta products bearing statements on the absence or reduced presence of gluten in accordance with the requirements of Commission Implementing Regulation (EU) No 828/2014[b]	60 g/kg	
Flavoured drinks	1.2 g/l	
Coffee, tea (excluding black tea), herbal and fruit infusions, chicory; tea, herbal and fruit infusions and chicory extracts; tea, plant, fruit and cereal preparations for infusions, as well as mixes and instant mixes of these products	9.6 g/l – the maximum level refers to the products ready to use	
Food supplements as defined in Directive 2002/46/EC[c], excluding food supplements for infants	3.0 g/day for general population 1.2 g/day for young children	

2' FL = 2'-fucosyllactose; UHT = ultra-high temperature.

a EUR-Lex, 2013. Regulation (EU) No 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control and repealing Council Directive 92/52/EEC, Commission Directives 96/8/EC, 1999/21/EC, 2006/125/EC and 2006/141/EC, Directive 2009/39/EC of the European Parliament and of the Council and Commission Regulations (EC) No 41/2009 and (EC) No 953/2009. Available online: https://www.legislation.gov.uk/eur/2013/609/contents.

b EUR-Lex, 2014. Commission Implementing Regulation (EU) No 828/2014 of 30 July 2014 on the requirements for the provision of information to consumers on the absence or reduced presence of gluten in food. Available online: https://www.legislation.gov.uk/eur/2014/828/contents.

c EUR-LEX, 2002. directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements. Available online: https://www.legislation.gov.uk/eudr/2002/46/contents.

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

As reported in previous EFSA opinions (EFSA NDA Panel, 2014; 2019a, 2019b, 2020b, 2020a, 2021, 2022a, 2022b) Human Milk Oligosaccharides (HMOs), including fucosyllactoses, are considered 'non-digestible oligosaccharides' (EFSA NDA Panel, 2014) since they do not undergo any significant digestion in the upper gastrointestinal tract (Brand-Miller et al., 1995, 1998; Chaturvedi et al., 2001; Engfer et al., 2000; Gnoth et al., 2000, 2001; Rudloff & Kunz, 2012).

Brand-Miller *et al*. (1995, 1998) reported that HMOs, consumed as a load (a purified oligosaccharide fraction from human milk), are fermented in the colon by the intestinal microbiota. Chaturvedi *et al*. (2001) and Coppa

et al. (2001) reported that 97% and 40–50%, respectively, of the ingested HMOs are excreted unchanged in faeces of breastfed infants. Furthermore, approximately 1–2% of the ingested amounts of HMOs is excreted unchanged in the infants' urine (EFSA NDA Panel, 2019b; Goehring et al., 2014; Kunz et al., 2017; Rudloff et al., 1996, 2006; Vazquez et al., 2017).

Based on information available on HMOs, it is considered that the NF does not undergo any significant digestion in the gastrointestinal tract and that only small amounts are expected to be absorbed. Moreover, there are no indications that the absorption of 2'-FL, which is the main constituent of the NF, or other structurally related mono- and oligosaccharides (e.g. D-lactose), differs from that of similar components found in human milk.

The ADME of human milk oligosaccharides are well understood and the information provided does not suggest HMOs produced by bio-fermentation would perform differently in the gut. As such the ADME information does not indicate any further areas of concern.

2.1.7. Nutritional Information

The novel food is mainly composed of the non-digestible oligosaccharide 2'-FL. The NF contains other carbohydrates individually present at low concentrations. Taking into account the composition of the NF and the proposed conditions of use, consumption of the NF is not considered to be nutritionally disadvantageous.

2.1.8. Toxicological Information

Toxicological proprietary studies were provided. The studies were conducted in accordance with Organisation for Economic Co-operation and Development (OECD) principles of Good Laboratory Practice (GLP) to support the safety assessment of the novel food. No human intervention studies with the NF were provided by the applicant and no reference to human data was made.

2.1.8.1. Genotoxicity

The potential genotoxicity of the NF was investigated in a bacterial reverse mutation test, an in vitro mammalian cell micronucleus test, an in vitro chromosomal aberration test in human lymphocytes and also in an in vivo micronucleus test in mice. The results of the studies are summarised in Table 3. As such the novel food was not considered genotoxic.

Table 3. Summary of Genotoxicity Data for 2'-FL

Reference	Type of study	Test system	Dose	Outcome
Study No. B18674 (Unpublished, 2019a)	Bacterial reverse mutation test (GLP, OECD	Salmonella Typhimurium TA98, TA100, TA1535,	313, 625, 1,250, 2,500 and 5,000 µg/ plate (absence	No reproducible or dose-related increases in revertant colony numbers over

Reference	Type of study	Test system	Dose	Outcome
	TG 471)	TA1537 and *E. coli* WP2uvrA (pKM101)	and presence of S9 mix)	control counts were observed with any of the strains following exposure to 2′-FL at any concentration.
Study No. B18675 (Unpublished, 2019b)	In vitro mammalian chromosomal aberration test (GLP, OECD TG 473)	Chinese Hamster Lung (CHL/IU) cells	1,250–5,000 µg/mL (absence and presence of S9 mix	The percentage of micronuclei was not significantly increased in any of the test substance concentrations.
Study No. 3267-292 (Unpublished, 2021)	In vitro human lymphocyte micronucleus test (GLP, OECD TG 487)	Blood from healthy donors	2,500–5,000 µg/mL (absence and presence of S9 mix)	The incidence of micro nucleated cells and the ratio of polychromatic erythrocytes to total erythrocytes in the NF treated groups was not statistically significantly different from the negative control group.
Study No. B18676 (Unpublished, 2019c)	In vivo micronucleus test (GLP, OECD TG 474)	Mouse, CrlOri:CD1(ICR),	2,500, 5,000, 7,500 mg/kg (oral administration via gastric intubation twice at 24-h intervals	A statistically significant increase in chromosomal aberrations was noted, but the increase was still within the historical control values of the laboratory. The NF did not induce other statistically significant increases in the frequency of chromosomal aberrations

2.1.8.2. Sub-chronic toxicity study

A 90-day oral toxicity study was performed according to the OECD Test Guideline 408 following GLP conditions, where groups of 10 neonatal Sprague–Dawley rats/sex/group (7-day old) were administered daily doses of 2,500, 5,000 and 7,500 mg per kg bw of 2′-FL in water by oral gavage. Additional rats (5 sex/dose) for the control and high dose groups were observed over a 4-week recovery period. Following the studies, the author of the study proposed the high dose level of 7,500 mg 2′-FL/kg bw as the no observed adverse effects level (NOAEL).

However, it was noted by the EFSA Panel that even in the absence of changes in bodyweight, food consumption and gross or histological changes, the highest dose of 7,500 mg 2′-FL/kg bw induced soft stool and diarrhoea in almost all animals of both sexes in the second and third month of treatment. The Panel therefore identified the intermediate dose of 5,000 mg 2′-FL/kg bw per day as the NOAEL (EFSA NDA Panel, 2022).

2.1.9. Allergenicity

The applicant did not identify any allergenic potential of introduced proteins as a result of the genetic modification of the *C. glutamicum* ATCC 13032 host strain according to the relevant opinion on the assessment of allergenicity (EFSA GMO Panel, 2010).

The criterion used for identifying allergenic proteins was that of considering 'higher than 35% identity in a sliding window of 80 amino acids. The protein content in the NF is low (≤ 0.005%) as indicated in the specifications.

The likelihood that the novel food will trigger allergic reactions in the target population under the proposed conditions of use was therefore considered to be low.

3. Other Regulators Opinions and Conclusions

EFSA (2022) found that this product, 2'-FL derived from genetically modified *Corynebacterium glutamicum* was safe under the proposed conditions of use.

2'-FL produced by either chemical synthesis or microbial fermentation using derivate strains of *Escherichia coli* K-12 (DH1) or *Escherichia coli* BL21 (DE3) is already authorised to be used in a number of food products including food supplements, with consumption expected to be safe.

The genotoxicity studies did not indicate any safety concerns. The sub-chronic toxicology studies indicated some effects on gastrointestinal transit at the highest doses of 7,500 mg 2'-FL/kg bw in the 90-Day Oral Toxicity Study in Rodents, the NOAEL of an intermediate dose of 5,000 mg 2'-FL/kg bw per day. Given humans are exposed to 2'-FL through breast milk and the novel food is identical to 2'-FL from other sources, the safe upper intake was set on the basis of infant exposure through breast milk.

Consumption of the novel food under the proposed conditions of use is not expected to be a safety concern as the NF would be consumed at the same extent as the already authorised 2'-FL. It was noted that breastfed infants are naturally exposed to these substances at the levels sought in the proposed uses.

4. Caveats and Uncertainties

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 NF as confirmed by NMR spectroscopy.
>
> (ii) information on the genetically modified production strain (DNA analysis and absence of viable cells).

5. FSA-FSS conclusion for GB safety assessment

The application has been assessed in line with the applicable guidance. The conclusions of the EFSA opinion have been reviewed in detail by the FSA and FSS and are considered appropriate and consistent within the identified caveats and uncertainties identified in the opinion and would be applicable to GB.

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 2'-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 following principles have been applied:

> 1) There is not a legal duty to perform a separate risk assessment for GB, there was 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.
>
> 2) Sufficient evidence was available in the literature, for example, where other National food safety authorities had positively assessed the application using the same risk assessment guidance in principle and legal requirements in GB with the exception to changes in the General Food Law.
>
> 3) Applicants provided sufficient relevant information as requested by FSA/FSS.

4) The FSA and FSS review did not find any issues of divergence from guidance or mutual approaches or new scientific issues for consideration.

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

Abbreviations

Abbreviation	Definition
2'-FL	2'-Fucosyllactose
3-FL	3'-Fucosyllactose
CAS	Chemical Abstracts Service
CrlOri:CD1(ICR)	Charles River Laboratories Orient: Cesarean-derived (Institute of Cancer Research)
DNA	Deoxyribonucleic acid
DFL	Difucosyllactose
DM	Dry matter
EC	European Commission
EFSA	European Food Safety Authority
EU	European Union
FSSC 22000	Food Safety System Certification 22000
FSA	Food Standards Agency
FSS	Food Standards Scotland
FS	Formula Supplements
GB	Great Britain
HACCP	Hazard Analysis Critical Control Point
HPAEC-PAD	High-performance anion-exchange chromatography–pulsed amperometric detection
HiMO	Human-identical milk oligosaccharide
IUPAC	International Union of Pure and Applied Chemistry
LC–MS/MS	Liquid chromatography–tandem mass spectrometry
OECD	Organisation for Economic Co-operation and Development
RP	Regulated Product
NOAEL	No observed adverse effect level
NF	Novel Food
NMR	Nuclear magnetic resonance spectroscopy
qPCR	Quantitative polymerase chain reaction
UK	United Kingdom
WGS	Whole genome sequence
W/W	Weight per weight

Submitted: December 04, 2024 GMT. Published: December 20, 2024 GMT.

References

Brand-Miller, J. C., McVeagh, P., McNeil, Y., & Gillard, B. (1995). Human milk oligosaccharides are not digested and absorbed in the small intestine of young infants. *Proceedings of the Nutrition Society of Australia*, *19*, 44.

Brand-Miller, J. C., McVeagh, P., McNeil, Y., & Messer, M. (1998). Digestion of human milk oligosaccharides by healthy infants evaluated by the lactulose hydrogen breath test. *The Journal of Paediatrics*, *133*, 95–98. https://doi.org/10.1016/S0022-3476(98)70185-4

Chaturvedi, P., Warren, C. D., Buescher, C. R., Pickering, L. K., & Newburg, D. S. (2001). Survival of human milk oligosaccharides in the intestine of infants. In D. S. Newberg (Ed.), *Bioactive components of human milk* (pp. 315–323). Springer Science + Business Media. https://doi.org/10.1007/978-1-4615-1371-1_39

Coppa, G. V., Pierani, P., Zampini, L., Bruni, S., Carloni, I., & Gabrielli, O. (2001). Characterization of oligosaccharides in milk and faeces of breast-fed infants by high-performance anion-exchange chromatography. *Advances in Experimental Medicine and Biology*, *501*, 307–314. https://doi.org/10.1007/978-1-4615-1371-1_38

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards). (2022). Statement on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 15: suitability of taxonomic units notified to EFSA until September 2021. *EFSA Journal*, *20*(1), 7045. https://doi.org/10.2903/j.efsa.2022.7045

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2018). Guidance on the characterisation of microorganisms used as feed additives or as production organisms. *EFSA Journal*, *16*(3), 5206. https://doi.org/10.2903/j.efsa.2018.5206

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms). (2010). Scientific opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. *EFSA Journal*, *8*(7), 1700. https://doi.org/10.2903/j.efsa.2010.1700EFSA

EFSA NDA Panel. (2022). Safety of 2′-fucosyllactose (2'-FL) produced by a derivative strain (APC199) of Corynebacterium glutamicum ATCC13032 as a novel food pursuant to Regulation (EU) 2015/2283. *EFSA Journal*, *20*(12). https://doi.org/10.2903/j.efsa.2022.7647

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies). (2014). Scientific opinion on the essential composition of infant and follow-on formulae. *EFSA Journal*, *12*(7), 3760. https://doi.org/10.2903/j.efsa.2014.3760

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies). (2016). Guidance on the preparation and presentation of an application for authorisation of a novel food in the context of Regulation (EU) 2015/2283. *EFSA Journal*, *14*(11), 4594. https://doi.org/10.2903/j.efsa.2016

EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens). (2019a). Scientific opinion on the safety of 2′-fucosyllactose/difucosyllactose mixture as a novel food pursuant to Regulation (EU) 2015/2283. *EFSA Journal*, *17*(6), 5717. https://doi.org/10.2903/j.efsa.2019.5717

EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens). (2019b). Scientific opinion on the safety of lacto-N-tetraose (LNT) as a novel food pursuant to Regulation (EU) 2015/2283. *EFSA Journal*, *17*(12), 5907. https://doi.org/10.2903/j.efsa.2019.5907

EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens). (2020a). Scientific opinion on the safety of 3'-sialyllactose (3'-SL) sodium salt as a novel food pursuant to Regulation (EU) 2015/2283. *EFSA Journal*, *18*(5), 6098. https://doi.org/10.2903/j.efsa.2020.6098

EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens). (2020b). Scientific opinion on the safety of lacto-N-neotetraose (LNnT) produced by derivative strains of E. coli BL21 as a novel food pursuant to Regulation (EU) 2015/2283. *EFSA Journal*, *18*(11), 6305. https://doi.org/10.2903/j.efsa.2020.6305

EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens). (2021). Scientific opinion on the safety of 3-FL (3-fucosyllactose) as a novel food pursuant to Regulation (EU) 2015/2283. *EFSA Journal*, *19*(6), 6662. https://doi.org/10.2903/j.efsa.2021.6662

EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens). (2022a). Scientific Opinion on the safety of 3-fucosyllactose (3-FL) produced by a derivative strain of Escherichia coli BL21 (DE3) as a Novel Food pursuant to Regulation (EU) 2015/2283. *EFSA Journal*, *20*(5), 7329. https://doi.org/10.2903/j.efsa.2022.7329

EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens). (2022b). Scientific Opinion on the safety of lacto-N-tetraose (LNT) produced by derivative strains of Escherichia coli BL21 (DE3) as a Novel Food pursuant to Regulation (EU) 2015/2283. *EFSA Journal*, *20*(5), 7242. https://doi.org/10.2903/j.efsa.2022.7242EFSA

EFSA Scientific Committee. (2007). Opinion of the Scientific Committee on a request from EFSA on the introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA. *EFSA Journal*, *5*(12), 587. https://doi.org/10.2903/j.efsa.2007

Engfer, M. B., Stahl, B., Finke, B., Sawatzki, G., & Daniel, H. (2000). Human milk oligosaccharides are resistant to enzymatic hydrolysis in the upper gastrointestinal tract. *The American Journal of Clinical Nutrition*, *71*, 1589–1596. https://doi.org/10.1093/ajcn/71.6.1589

EU (European Union). (2015). *Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001*. https://www.legislation.gov.uk/eur/2015/2283/contents

EU (European Union). (2017). *Commission Implementing Regulation (EU) 2017/2469 of 20 December 2017 laying down administrative and scientific requirements for applications referred to in Article 10 of Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods*. https://www.legislation.gov.uk/eur/2017/2469/contents

Gnoth, M. J., Kunz, C., Kinne-Saffran, E., & Rudloff, S. (2000). Human milk oligosaccharides are minimally digested in vitro. *The Journal of Nutrition*, *130*, 3014–3020. https://doi.org/10.1093/jn/130.12.3014

Gnoth, M. J., Rudloff, S., Kunz, C., & Kinne, R. K. (2001). Investigations of the in vitro transport of human milk oligosaccharides by a Caco-2 monolayer using a novel high-performance liquid chromatography-mass spectrometry technique. *Journal of Biological Chemistry*, *276*, 34363–34370. https://doi.org/10.1074/jbc.M104805200

Goehring, K. C., Kennedy, A. D., Prieto, P. A., & Buck, R. H. (2014). Direct evidence for the presence of human milk oligosaccharides in the circulation of breastfed infants. *PLoS One*, *9*, e101692. https://doi.org/10.1371/journal.pone.0101692

Israel MOH (Israel Ministry of Health). (2019). *[Updated novel food guidance on: Oligosaccharide 2'-Fucosyllactose of September 10 2019]*. Ministry of Health Israel (MOH), Food Control Services, Public Health Services, Tel Aviv, State of Israel. https://www.health.gov.il/UnitsOffice/HD/PH/FCS/NovelFood/Pages/NovelFoodList.aspx

Kunz, C., Meyer, C., Collado, M. C., Geiger, L., Garcıa-Mantrana, I., Bertua-Rıos, B., Martınez-Costa, C., Borsch, C., & Rudloff, S. (2017). Influence of gestational age, secretor, and Lewis blood group status on the oligosaccharide content of human milk. *JPGN*, *64*, 789–798. https://doi.org/10.1097/MPG.0000000000001402

Rudloff, S., & Kunz, C. (2012). Milk oligosaccharides and metabolism in infants. *American Society for Nutrition. Advances in Nutrition*, *3*, 398S-405S. https://doi.org/10.3945/an.111.001594

Rudloff, S., Obermeier, S., Borsch, C., Pohlentz, G., Hartmann, R., Breosicke, H., Lentze, M. J., & Kunz, C. (2006). Incorporation oforally applied (13)C-galactose into milk lactose and oligosaccharides. *Glycobiology*, *16*, 477–487. https://doi.org/10.1093/glycob/cwj092

Rudloff, S., Pohlentz, G., Diekmann, L., Egge, H., & Kunz, C. (1996). Urinary excretion of lactose and oligosaccharides inpreterm infants fed human milk or infant formula. *Acta Paediatrica*, *1996*, 598–603. https://doi.org/10.1111/j.1651-2227.1996.tb14095.x

Vazquez, E., Santos-Fandila, A., Buck, R., Rueda, R., & Ramirez, M. (2017). Major human milk oligosaccharides are absorbed into the systemic circulation after oral administration in rats. *British Journal of Nutrition*, *117*, 237–247. https://doi.org/10.1017/S0007114516004554