REGULATED PRODUCTS SAFETY ASSESSMENT

Assessment of a Modification of Specifications of 2'-Fucosyllactose (2'-FL) Produced by a Genetically Modified Strain of *Escherichia Coli* BL21 (RP2257)

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

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

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FSA Research and Evidence

The Food Standards Agency (FSA) and Food Standards Scotland (FSS), in August 2024, received an application from Chr. Hansen A/S, Denmark ('the applicant') for a change to the conditions of use of the novel food 2'-fucosyllactose (2'-FL), produced by a genetically modified strain of *Escherichia coli* BL21 (DE3).

2'-FL has been the subject of several prior applications in the EU, under the previous novel food Regulation (EC) 258/97 and assimilated Regulation (EU) 2015/2283 applicable to GB. 2'-FL is currently authorised for use in several food and drinks categories for the general population and for infants and young children, including infant formula and follow-on formula.

This application seeks to harmonise the specifications of 2'-FL with other authorised human milk oligosaccharides, including synthetic 2'-FL and 2'-FL produced by other organisms, as included in the Great Britain register of novel foods.

The FSA and FSS concluded that the applicant had provided sufficient information to assure that the change in the conditions of use of 2'-FL, produced by a genetically modified strain of *E. coli* BL21, is safe under the intended conditions of use.

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

This is a joint FSA and FSS publication.

1. Introduction

2′-Fucosyllactose (2′-FL) is an authorised novel food in the UK (assimilated Regulation (EU) 2017/2470). In accordance with assimilated Regulation (EU) 2015/2283 on novel foods, the application RP2257 was submitted to the three nations of Great Britain (GB) for a change to the specifications of 2′-fucosyllactose, produced using a genetically modified strain of *Escherichia coli* BL21 (DE3), to increase the permitted level of endotoxins from ≤0.1 EU (endotoxin units)/mg to ≤10 EU/mg in powder form and from ≤0.1 EU/µL to ≤10 EU/µL in liquid form. The applicant, Chr. Hansen A/S (Denmark), is seeking this authorisation to harmonise the specifications of 2′-FL produced using *E. coli* BL21 with the specifications of synthetic 2′-FL, 2′-FL produced by other organisms, and other human milk oligosaccharides (HMOs) already authorised in GB.

2′-FL is a non-digestible HMO which occurs in high abundance in human breast milk (Urashima et al., 2022). 2′-FL is not a new novel food ingredient; 2′-FL, from chemical or microbial sources, is a currently authorised novel food in GB under assimilated Regulation (EU) 2017/2470. 2′-FL manufactured by microbial fermentation using a genetically modified strain of *E. coli* BL21 was authorised in the EU (and UK) in 2017 following an opinion through the 'initial assessment' process of Regulation (EC) 258/97 by the Medicines Evaluation Board Agency of the Netherlands (MEBA, 2016). Use of 2′-FL, produced by a genetically modified strain of *E. coli* K-12 (DH1), is also authorised in GB, alone or in mixture with difucosyllactose (DFL) (assimilated Regulations (EU) 2019/388 and 2019/1979).

A change in conditions of use of 2′-FL produced by *E. coli* BL21, for its inclusion in infant formula and follow-on formula at a higher use level, has been positively assessed by the European Food Safety Agency (EFSA NDA Panel, 2023c) and the FSA/FSS (FSA & FSS, 2025).

In October 2023, the applicant submitted an application to the European Commission to change the specifications of 2′-FL produced using *E. coli* BL21, to increase the permitted level of endotoxins. This change was authorised in July 2024 in without undertaking a risk assessment (Commission Implementing Regulation (EU) 2024/2102).

The FSA and FSS have undertaken a safety assessment of the proposed change to conditions of use of 2'-FL under the novel foods legislation, assimilated Regulation (EU) 2015/2283.

The evaluation by the FSA and FSS assessed the food safety risks of the novel food and its production, in line with Article 11 of assimilated Regulation (EU) 2015/2283 and Article 7 of assimilated Regulation (EU) 2017/2469. The basis and structure of the assessment was conducted in accordance with the relevant technical guidance adopted by the FSA and FSS full novel food applications (EFSA NDA Panel, 2016).

Under Article 3(4) of assimilated Regulation (EU) 2017/2469, it may not be necessary for the applicant to provide all the data required under Article 5 of this Regulation when a novel food application seeks to modify the conditions of use, the specifications, additional specific labelling requirements or post-market monitoring requirements of an authorised novel food. Verifiable justification explaining that the intended changes do not affect the results of the existing safety assessment is provided by the applicant. Given that the novel food is identical to the one reviewed in the previous assessment, this new safety assessment has focused on the impact of the intended change of specification for the novel food.

As the identity of the novel food and its production process remain unchanged, the conclusions drawn from the information provided in the original application on the identity of the novel food; production process; stability; proposed use and anticipated intake; absorption, distribution, metabolism and excretion (ADME); toxicity; and allergenicity, would not be affected by the proposed change in specifications. Therefore, this information was not provided or reviewed as part of the assessment.

This assessment outlines the conclusions of the FSA and FSS on the change in the conditions of use for 2'Fl produced using *E. coli* BL21 to amend the specification of the novel food.

This assessment represents the opinion of the FSA and FSS.

2. Assessment

2.1. Identity of the novel food

The novel food is 2'-fucosyllactose (2'-FL), a trisaccharide consisting of L-fucose, D-galactose and D-glucose, produced with a genetically modified strain of *E. coli* BL21 (DE3), in both powdered and liquid concentrate form. There is no change to the identity of the novel food as currently defined in the Union list of novel foods (assimilated Implementing Decision (EU) 2017/2201).

The applicant provided compositional data for five independent batches of the product, which was reviewed by the FSA/FSS. The identity of the novel food is not changed by the current application made to the FSA and FSS, and as such has not been subject to further assessment.

2.2. Production process

The applicant stated that the production process of the novel food 2'-FL in the current application is identical to the production process of 2'-FL produced by microbial fermentation by a genetically modified strain of *E. coli* BL21 (DE3), as authorised in assimilated Implementing Decision (EU) 2017/2201.

The unchanged production process has not been subject to further assessment.

2.3. Compositional information

The applicant stated that the composition for 2'-FL produced with a genetically modified strain of *E. coli* BL21 (DE3) is unchanged from the composition as assessed and authorised in assimilated Implementing Decision (EU) 2017/2201. The FSA/FSS requested compositional data from five independent representative batches of the product, to assure that the composition of the novel food is not changed by the current application made to the FSA and FSS. The FSA/FSS reviewed the compositional data provided by the applicant and confirmed that the final product is free of the production organism and of any recombinant genetic material and mainly consists of the substance 2'-FL and minor portions of other carbohydrate byproducts.

The composition of the novel food has therefore not been subject to further assessment.

2.4. Stability

Information concerning the stability of the novel food was assessed in the original authorisation and did not raise concerns (MEBA, 2016). No further information concerning the stability of the novel food was given in the current application.

The FSA and FSS conclude that the changes of specification sought by the applicant do not change the original opinion on stability.

2.5. Specifications

The applicant requested a change in the specifications of the novel food, to increase the permitted level of endotoxins from ≤0.1 EU (endotoxin units)/mg to ≤10 EU/mg in powder form and from ≤0.1 EU/µL to ≤10 EU/

µL in liquid form (Table 1). The applicant seeks this authorisation to harmonise the specifications of 2′-FL produced using *E. coli* BL21 with the specifications of synthetic 2′-FL, 2′-FL produced by other organisms, and other human milk oligosaccharides (HMOs) already authorised in GB.

Table 1. Proposed change in endotoxin specification for 2′-fucosyllactose produced with a genetically modified strain of *E. coli* BL21 (DE3).

Product type	Current specification	Proposed specification	Method
Powder	≤0.1 EU/mg (≤100 EU/g)	≤10 EU/mg	Ph.Eur. 2.6.14
Liquid concentrate	≤0.1 EU/µL (≤100 EU/mL)	≤10 EU/µL	Ph.Eur. 2.6.14

EU: endotoxin units.

As one of the first HMOs to be authorised as a novel food, 2′-FL was assessed before there were any standard specifications for HMOs; therefore a cautious provisional endotoxin limit of ≤0.1 EU/mg (≤100 EU/g) was adopted. The applicant's proposed new endotoxin specification (≤10 EU/mg) has subsequently been authorised in the EU and GB, based on EFSA assessments, for 2′-FL produced by chemical synthesis (EFSA NDA Panel, 2015, assimilated Regulation (EU) 2017/2470) or using *E. coli* K-12 (assimilated Regulation (EU) 2019/388), and has been positively assessed by EFSA using *E. coli* W (EFSA NDA Panel, 2023a), without raising any safety concerns. 2′-FL is also authorised in GB in combination with other HMOs (DFL and lacto-*N*-fucopentaose I (LNFP-I), assimilated Regulation (EU) 2017/2470) with an endotoxin specification of ≤10 EU/mg.

The applicant has also successfully applied for authorisation of a range of other HMOs with an endotoxin specification of ≤10 EU/mg, in the EU (3-fucosyllactose (3-FL) (EFSA NDA Panel, 2022b); 3′-sialyllactose (3′-SL) (EFSA NDA Panel, 2022c); 6′-sialyllactose (6′-FL) (EFSA NDA Panel, 2022d); lacto-*N*-tetraose (LNT) (EFSA NDA Panel, 2022e)) and GB (3-FL (FSA & FSS, 2024a)). Based on the data provided by the applicant, no safety concerns were identified.

In 2024, the European Commission implemented the applicant's proposed change in endotoxin specification for 2′-FL produced by *E. coli* BL21, stating that the change is "not liable to have an effect on human health" (Commission Implementing Regulation (EU) 2024/2102).

It is noted that none of the previous assessments have explained why the level of endotoxins was considered safe at 10 EU/mg. However, each opinion reached this conclusion in the context of consumption by vulnerable groups and understanding of the level of consumption by infants and young children, and that no concerns were raised. It is also noted that products aimed at infants will also need to be compliant with relevant regulations for those products.

The FSA/FSS requested compositional analysis from five independent representative batches of the product, to assure that the current production of the product met the proposed specifications. The five batches, produced between 2024 and 2025, all showed endotoxin levels <5 EU/g (<0.005 EU/mg), demonstrating that the novel food can be consistently produced to the proposed specification (Table 2).

Table 2. Endotoxin analysis of five independent batches of 2′-fucosyllactose produced with a genetically modified strain of *E. coli* BL21 (DE3).

Current specification	Method	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5
≤0.1 EU/mg (≤100 EU/g)	Ph. Eur. 2.6.14	<5 EU/g	<5 EU/g	<5 EU/g	<5 EU/g	<5 EU/g

EU: endotoxin units.

The FSA and FSS therefore conclude that the applicant's proposed change in endotoxin specification from ≤0.1 EU/mg (≤100 EU/g) to ≤10 EU/mg for 2′-FL produced by *E. coli* BL21 does not raise any safety concerns. The revised specification for the product is detailed in Table 3 (powder form) and Table 4 (liquid form).

Table 3. Revised specification of 2′-fucosyllactose produced with a genetically modified strain of *E. coli* BL21 (DE3) (powder form).

		Unit	Specification	Method
Identified carbohydrates	2'-Fucosyllactose	%	≥90	HPAEC-PAD
	Lactose	%	≤5	
	3-Fucosyllactose	%	≤5	
	Difucosyllactose	%	≤5	
	Fucosylgalactose	%	≤3	
	Glucose	%	≤3	
	Galactose	%	≤3	
	Fucose	%	≤3	
	Water	%	≤9	Karl-Fischer titration
	Protein	%	≤0.01	Nanoquant
	Ash	%	≤0.5	ASU L 06.00-4 (a)
Heavy Metals	Arsenic	mg/kg	≤0.2	ASU L 12.06-6 (a)
	Cadmium	mg/kg	≤0.1	ASU L 00.00-19/3 8 (a)
	Lead	mg/kg	≤0.02	ASU L 00.00-19/3 8 (a)
	Mercury	mg/kg	≤0.5	ASU L 00.00-19/4 (a)
	Endotoxins	EU/mg	≤10	Ph.Eur. 2.6.14
	Aflatoxin M_1	µg/kg	≤0.025	DIN EN ISO 14501
Microbiology	Standard plate count	CFU/g	≤10^4	ISO 4833-2
	Yeast and mould	CFU/g	≤100	ISO 21527-2
	Coliform / Enterobacteriaceae	CFU/11 g	Absent	ISO 4832/ISO 21528-2

		Unit	Specification	Method
	Salmonella spp.	CFU/100 g	Absent	ISO 6579
	Cronobacter	CFU/100 g	Absent	ISO/TS 22964

HPAEC-PAD: high-performance anion-exchange chromatography with pulsed amperometric detection; EU: endotoxin unit; CFU: colony-forming unit.

Table 4. Revised specification of 2′-fucosyllactose produced with a genetically modified strain of *E. coli* BL21 (DE3) (liquid form).

		Unit	Specification	Method
Identified carbohydrates	2'-Fucosyllactose	%	≥90	HPAEC-PAD
	Lactose	%	≤5	
	3-Fucosyllactose	%	≤5	
	Difucosyllactose	%	≤5	
	Fucosylgalactose	%	≤3	
	Glucose	%	≤3	
	Galactose	%	≤3	
	Fucose	%	≤3	
	Protein	%	≤0.01	Nanoquant
	Ash	%	≤0.5	ASU L 06.00-4 (a)
Heavy Metals	Arsenic	mg/kg	≤0.2	ASU L 12.06-6 (a)
	Cadmium	mg/kg	≤0.1	ASU L 00.00-19/3 8 (a)
	Lead	mg/kg	≤0.02	ASU L 00.00-19/3 8 (a)
	Mercury	mg/kg	≤0.5	ASU L 00.00-19/4 (a)
	Endotoxins	EU/µL	≤10	Ph.Eur. 2.6.14
	Aflatoxin M_1	µg/kg	≤0.025	DIN EN ISO 14501
Microbiology	Standard plate count	CFU/g	≤5000	ISO 4833-2
	Yeast and mould	CFU/g	≤50	ISO 21527-2
	Coliform / Enterobacteriaceae	CFU/11 g	Absent	ISO 4832/ISO 21528-2
	Salmonella spp.	CFU/200 mL	Absent	ISO 6579
	Cronobacter	CFU/200 mL	Absent	ISO/TS 22964

HPAEC-PAD: high-performance anion-exchange chromatography with pulsed amperometric detection; EU: endotoxin unit; CFU: colony-forming unit.

2.6. History of Use

2′-FL is one of several oligosaccharides which occurs naturally in mammalian milk. It is present in especially high abundance in human milk (Urashima et al., 2022), with the concentration varying between individuals and over time during lactation (Lagström et al., 2020; Lefebvre et al., 2020; Samuel et al., 2019).

2′-FL, produced by synthesis or by fermentation from derivative strains of *E. coli* BL21 or *E. coli* K-12, is already authorised in Great Britain from retained EU law (assimilated Regulation (EU) 2017/2470). 2′-FL produced

using a derivative strain of *C. glutamicum* has received a positive opinion in the EU (EFSA NDA Panel, 2022a) and in GB (FSA & FSS, 2024b). 2'-FL produced from *E. coli* BL12 is also authorised as a food ingredient in Canada (Health Canada, 2020), the USA (through the Generally Regarded as Safe (GRAS) scheme: GRN 571 (FDA, 2015); GRN 929 (FDA, 2021); GRN 1014 (FDA, 2022)), Israel (Israel MOH, 2019), and Australia and New Zealand (FSANZ, 2021).

2.7. Proposed Use and Anticipated Intake

2'-FL as a novel food is already authorised in the EU and GB. The current application does not request a change in the proposed uses and use levels. This section has therefore not been subjected to further assessment.

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

2'-FL as a novel food is already authorised in the EU and GB. ADME was assessed as part of the original novel food assessment, and existing conclusions on the safety of the novel food itself remain valid.

The ADME of 2'-FL was therefore not further assessed by the FSA and FSS in this application.

2.9. Nutritional Information

The novel food is primarily composed of the non-digestible oligosaccharide 2'-FL, which is structurally identical to the naturally occurring counterpart in human breast milk. The requested change to the specifications of 2'-FL does not change the nutritional value of the food.

The nutritional information of 2'-FL was therefore not further assessed by the FSA and FSS in this application.

2.10. Toxicological Information

2'-FL is already authorised as a novel food in the EU and GB. Toxicity was assessed as part of the original novel food assessment and did not raise any concerns at the proposed use and anticipated intake.

The applicant's proposed endotoxin specification (≤10 EU/mg) has already been assessed for 2'-FL produced using *E. coli* W (EFSA NDA Panel, 2023a), and 2'-FL produced using *E. coli* K-12 in combination with other HMOs (2'-FL/DFL (EFSA NDA Panel, 2019); LNFP-I/2'-FL: (EFSA NDA Panel, 2023b)), without raising any toxicological concerns. This endotoxin specification has also been positively assessed for other HMOs (3-FL (EFSA NDA Panel, 2022b; FSA & FSS, 2024a); 3'-SL (EFSA NDA Panel, 2020, 2022c); 6'-SL (EFSA NDA Panel, 2022d); LNT (EFSA NDA Panel, 2022e)).

The FSA and FSS therefore conclude that the change to the endotoxin specification will not affect the existing conclusions on the toxicological safety of the novel food.

2.11. Allergenicity

2'-FL as a novel food is already authorised in the EU and GB. Allergenicity was assessed as part of the original novel food assessment and existing conclusions on the safety of the novel food itself remain valid. The change in endotoxin specification does not alter the conclusions on allergenicity; therefore, allergenicity was not further reviewed in this assessment.

3. Discussion

The novel food is 2'-fucosyllactose (2'-FL), a human milk oligosaccharide already authorised in GB, produced using a genetically modified strain of *E. coli* BL21 (DE3). The applicant provided compositional data to confirm that the novel food under production is identical to that originally authorised. The applicant requested a change in the endotoxin specifications for the novel food, from ≤0.1 EU/mg (≤100 EU/g) to ≤10 EU/mg in powder form and from ≤0.1 EU/µL (≤100 EU/mL) to ≤10 EU/µL in liquid form. This proposed specification has been positively assessed for 2'-FL produced by chemical synthesis and various other *E. coli* strains, without raising any safety concerns, and has been authorised for 2'-FL produced using *E. coli* BL21 in the European Union (Commission Implementing Regulation (EU) 2024/2102).

The FSA and FSS reviewed the original authorisation for 2'-FL produced using *E. coli* BL21 (DE3), and the safety assessments of 2'-FL and other HMOs which are in the public domain, in forming the opinion that the proposed change to the endotoxin specification does not raise any safety concerns.

4. Conclusions

The application has been assessed 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 Implementing Regulation (EU) 2017/2469 for the purposes of the GB assessment.

The FSA and FSS have undertaken the assessment of the change to the conditions of use of 2'-fucosyllactose (2'-FL) produced by genetically modified *E. coli* BL21 (DE3) and concluded that the novel food is safe and does not pose a safety risk to human health. The change in specification to increase the level of residual endotoxins was reviewed and does not pose a safety concern.

These conclusions were based on the information in the novel food dossier and the supplementary information submitted by the applicant.

Abbreviations

Abbreviation	Definition
2'-FL	2'-fucosyllactose
3-FL	3-fucosyllactose
3'-SL	3'-sialyllactose
6'-SL	6'-sialyllactose
ADME	Absorption, distribution, metabolism and excretion
CFU	Colony-forming unit
DFL	Difucosyllactose
EU	Endotoxin units
EFSA	European Food Safety Authority
EFSA NDA Panel	EFSA Panel on Dietetic Products, Nutrition and Allergies
FDA	Food and Drug Administration
FSA	Food Standards Agency
FSANZ	Food Standards Australia and New Zealand
FSS	Food Standards Scotland
GB	Great Britain
GRAS	Generally Regarded as Safe
HMO	Human milk oligosaccharide
HPAEC-PAD	High-performance anion-exchange chromatography with pulsed amperometric detection
LNFP-I	Lacto-*N*-fucopentaose I
LNT	Lacto-*N*-tetraose
MEB	Medicines Evaluation Board Agency
MOH	Israel Ministry of Health

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