Meeting

2’-Fucosyllactose from Kyowa Hakko Bio Co Ltd Discussion Paper

Committee Paper for Discussion - ACNFP/162/09

Advisory Committee For Novel Foods and Processes.

Application for an extension of use of 2’-Fucosyllactose, from Kyowa Hakko Bio Co Ltd

Application number RP1476

Issue

1. An application has been received under the novel food authorisation process (regulation 2015/2283) for an extension of use of the authorised novel food 2’-fucosyllactose (2’-FL), a Human Milk Oligosaccharide (HMO). The application seeks to change the existing authorisation for 2’Fucosyllactose in three ways: to use an alternative microbial production organism, a change to the specifications and for extended uses in food supplements for infants.

2. The Committee is asked to advise whether the available data provides an adequate basis for a risk assessment, and whether the novel food, as changed by the extension of use application, is safe and not nutritionally disadvantageous. Members are asked to focus on the changes sought to the current authorisations per the retained Union list and the additional evidence provided.

Background

3. On the 10th March 2022 the FSA and FSS received the submission for the extension of use for 2’-FL, an authorised novel food under Commission Implementing Regulation (EU) 2017/2470, from Kyowa Hakko Bio Co., Ltd. The application seeks authorisation for use of a genetically modified derivative strain of E. coli W in the fermentation process, a change to the specification parameter

levels for 2’-FL and to extend permitted uses to food supplements for infants. The 2’-FL being sought for authorisation has a lowered purity by specification of ≥82 % (dry weight basis (dwb)), compared to current authorisations.

4. The applicant seeks to market their 2’-FL novel food on the GB market for the first time. Prior authorisations under Commission Implementing Regulation (EU) 2017/2470 relate to other applicants but are open to all producers where their product meets the specification as there are no data protection provisions in place.

5. The novel food ingredient is produced via fermentation using a genetically modified strain of E. coli W followed by a series of purification steps involving isolation, concentration, and spray-drying. The applicant proposes to use the novel food in the current authorised uses for 2’-FL namely conventional foods and beverages, foods for specific groups (including FSMPs, total diet replacement for weight control, infant formulas, follow-on formula and foods for infants and young children) including food supplements (extended for use in infants), as alternative sources of 2’-FL and in the absence of other exposure sources.

6. The application dossier is attached as Annex A. The request for further information letter (with responses) is attached as Annex B. All supporting documents for all sections of the application, which have been updated following the request for further information, can be found in Annex C. All annexes contain confidential information.

7. As part of a trial for a new way of working for some applications, a draft Committee Advice Document for this application has been prepared for members consideration, alongside this introductory paper. This reflects that there have been several reviews for breast milk sugars and as such the key considerations have been discussed by the Committee in the past. Members are invited to review and comment on the draft found in Annex D.

This application

Identification

8. The applicant states that the identity of 2’-FL is structurally and chemically identical to the authorised novel food, 2’-FL.

9. The novel food ingredient, 2’-FL, is a fucosylated oligosaccharide composed of three molecules L-fucose, D-galactose, and D-glucose and is identical in structure

to 2’-FL found in human milk (Annex A; p13 – 15 of dossier). Purity by specification is ≥82% (dry weight basis (dwb)) and the final novel food product includes low levels of related saccharide molecules D-lactose (≤5% w/w), fucosylgalactose (≤3% w/w), difucosyllactose (≤3% w/w), L-fucose (≤1% w/w), D-glucose and D-galactose (≤1% w/w) by specification. Identity confirmed by LC-MS spectrometry, comparative NMR spectroscopy and 1H NMR and 13C NMR spectroscopy (Annex C [Annex A). The final composition is verified by HPLC-PAD (Annex C [Annex G]).

10. The applicant has provided a range of data that characterises the identity of the genetically modified fermentation microorganism E. coli W, including taxonomic classification (Annex A; p 15 and Table 2.a.2-1 of dossier) and information on the modification and donor organisms (Annex C [Annex B]). Identity verified by Whole Genome Sequence (WGS) and concluded highly homologous to the E. coli host strain per the American Type Culture Collection (ATCC) ATCC 9637 (Annex C [Annex C]).

Production Process

11. The extension of use application proposes an alternative microbial source for production of 2’-FL using a genetically modified strain of E.coli W. Prior authorisations have utilised genetically modified strains of Escherichia coli K-12 or E. coli BL21, as defined in the retained Union list.

12. The production method is described in detail (Annex A; p20 of dossier) and a schematic overview is provided (Annex A; p 21 of dossier).

13. The production process involves a 2-step fermentation and purification process. The initial fermentation step involves culture of the production microorganism in a defined nutrient media under controlled conditions. Main fermentation involves inoculation of the cultured E. coli W into a nutrient media to synthesise 2'-FL in the presence of glucose and lactose.

14. The second processing step involves isolation and purification of 2’-FL using filtration and cationic and anionic exchange followed by concentration and spray-drying. Additional details on pH and controls were provided by the applicant (Annex B: p 3 of response letter)

15. The acceptance criteria and specifications for the raw materials and processing aids have been provided (Annex A; p 16 – 19 and Table 2.b.1.2-1 of dossier) (Annex C [Annex D]). More details on the altered nutrient media, that

removes the use of soy peptone had been requested which the applicant considered with respect to any change any to parameters or hazards in the production process. The applicant states that the additional raw materials do not introduce new hazards or changes to parameters in the production process (Annex B; p 2 of response letter).

16. The applicant has provided a HACCP Plan (Annex C [Annex E]) to include critical control measures alongside justifications. Quality control is described (Annex A; p 21 of dossier).

Composition

17. The applicant has reported analytical data for a number of independent batches of 2’-FL (Annex A; p 22 – 26 and Table 2.c.2.1-1 of dossier) (Annex C [Annex F]). Additional analyses were requested to provide comparable data for all parameters on batches representative of the final product and as intended to be manufactured (Annex B; p 4 - 9 and Table 3.a-1 of response letter). The data indicates that the manufacturing process results in a consistent final ingredient that meets the newly proposed specifications, under the extension of use application, for the novel ingredient – see Tables 1- 6 below. The CoA’s are provided in Annex C [Annex F].

Table 1. Compositional batch analysis of 2’-FL manufactured with a fermentation media containing soy peptone: physicochemical properties, carbohydrate content and purity.

		Batch A	Batch B	Batch C	Batch D	Batch E	Batch F
Parameter	Specification						
Physicochemical							
pH	4.0 – 9.0	6.3	6.4	6.2	5.7	6.1	6.2

Composition							
Purity (2’-FL)	≥82 (% dwb)	92	92	92	91	96	94
D-lactose	≤5 w/w %	3.1	2.7	2.3	2.4	2.1	2.3
L-fucose	≤1 w/w %	≤0.05 (LOQ)	0.1	0.1	0.1	0.1	0.1
Fucosylgalactose	≤3 w/w %	0.8	0.5	0.4	0.9	0.1	0.8
Difucosyllactose (difucosyl-d-lactose)	≤3 w/w %	0.5	1.4	0.9	1.0	1.1	1.0
D-glucose and D-galactose	≤1 w/w %	0.2	0.1	0.1	0.2	≤0.05 (LOQ)	0.1
Sum of other carbohydrates (2)	% dwb	3.15	3.1	4.1	4.4	0.45	1.6
Water	≤9.0 w/w %	5.0	3.9	3.9	2.7	2.8	2.3
Ash	≤0.5 w/w%	0.2	0.1	0.1	0.03 ≤(LOQ)	0.1	0.1
Residual protein (internal method)	≤10 mg/kg (0.001%)	≤1 (LOQ)	≤1 (LOQ)	≤1 (LOQ)	≤1 (LOQ)	≤1 (LOQ)	≤1 (LOQ)
Residual protein (Bradford assay)	≤100 mg/kg (1)	NT	NT	NT	NT	NT	NT

2’-FL: 2’-fucosyllactose; dwb: dry weight basis; NT: not tested; LOQ: limit of quantification. (1) Evaluated using a test limit at 100 ppm; (2) Sum of

carbohydrates such as D-lactose, L-fucose, Fucosylgalactose, Difucosyllactose (difucosyl-d-lactose) and D-glucose and D-galactose.

Table 2. Compositional batch analysis of 2’-FL manufactured with an updated fermentation media without soy peptone: physicochemical properties, carbohydrate content and purity.

		Batch H	Batch I	Batch J
Parameter	Specification			
Physicochemical				
pH	4.0 – 9.0	6.1	6.0	6.2
Composition				
Purity (2’-FL)	≥82 (% dwb)	94	93	91
D-lactose	≤5 w/w %	1.1	1.1	1.1
L-fucose	≤1 w/w %	≤0.05	≤0.05	≤0.05
Fucosylgalactose	≤3 w/w %	1.0	1.0	1.0
Difucosyllactose (difucosyl-d-lactose)	≤3 w/w %	0.4	0.4	0.4
D-glucose and D-galactose	≤1 w/w %	0.3	0.3	0.3

Sum of other carbohydrates (2)	≤10 (% dwb)	3.1	4	6.1
Water	≤9.0 w/w %	4.7	5.3	4.5
Ash	≤0.5 w/w%	0.1	0.2	0.1
Residual protein (internal method)	≤10 mg/kg (0.001%)	NT	NT	NT
Residual protein (Bradford assay)	≤100 mg/kg (1)	≤100	≤100	≤100

2’-FL: 2’-fucosyllactose; dwb: dry weight basis; NT: not tested. (1) Evaluated using a test limit at 100 ppm; (2) Sum of carbohydrates such as D-lactose, L-fucose, Fucosylgalactose, Difucosyllactose (difucosyl-d-lactose) and D-glucose and D-galactose.

Table 3. Compositional batch analysis of 2’-FL manufactured with a fermentation media containing soy peptone: heavy metal contaminants.

		Batch A	Batch B	Batch C	Batch D	Batch E	Batch F
Parameter	Specification						
Heavy metals:							
Arsenic	≤0.2 mg/kg	≤0.05 (LOQ)	≤0.05 (LOQ)	≤0.05 (LOQ)	≤0.05 (LOQ)	≤0.05 (LOQ)	≤0.05 (LOQ)
Cadmium	≤0.2 mg/kg	≤0.05 (LOQ)	≤0.05 (LOQ)	≤0.05 (LOQ)	≤0.05 (LOQ)	≤0.05 (LOQ)	≤0.05 (LOQ)

Lead	≤0.2 mg/kg	≤0.05 (LOQ)	≤0.05 (LOQ)	≤0.05 (LOQ)	≤0.05 (LOQ)	≤0.05 (LOQ)	≤0.05 (LOQ)
Mercury	≤0.2 mg/kg	≤0.05 (LOQ)	≤0.05 (LOQ)	≤0.05 (LOQ)	≤0.05 (LOQ)	≤0.05 (LOQ)	≤0.05 (LOQ)
Iron	≤10 mg/kg	0.1	≤0.05 (LOQ)	≤0.05 (LOQ)	≤0.05 (LOQ)	≤0.05 (LOQ)	≤0.05 (LOQ)

LOQ: limit of quantification

Table 4. Compositional batch analysis of 2’-FL manufactured with an updated fermentation media without soy peptone: heavy metal contaminants.

		Batch H	Batch I	Batch J
Parameter	Specification			
Heavy metals:				
Arsenic	≤0.2 mg/kg	0.01	0.01	0.01
Cadmium	≤0.2 mg/kg	0.01	0.01	0.01
Lead	≤0.2 mg/kg	0.02	0.02	0.02
Mercury	≤0.2 mg/kg	0.004	0.004	0.004
Iron	≤10 mg/kg	0.96	0.10	0.70

LOQ: limit of quantification

Table 5. Compositional batch analysis of 2’-FL manufactured with a fermentation media containing soy peptone: microbial contaminants.

		Batch A	Batch B	Batch C	Batch D	Batch E	Batch F
Parameter	Specification						
Microbial parameters:							
Aerobic plate count	≤1,000 CFU/g	40	40	10	110	10	10
Moulds and Yeasts	≤100 CFU/g	100	100	100	100	100	100
Salmonella	Absent in 100 g	Negative	Negative	Negative	Negative	Negative	Negative
Enterobacteriaceae	Absent in 10 g	Negative	Negative	Negative	Negative	Negative	Negative
Cronobacter spp. (*Enterobacter sakazakii*)	Absent in 100 g	Negative	Negative	Negative	Negative	Negative	Negative
Listeria monocytogenes	Absent in 25 g	Negative	Negative	Negative	Negative	Negative	Negative
Bacillus cereus	≤50 CFU/g	10	10	10	10	10	10
Residual endotoxins	≤10 EU/mg	0.0092	0.0005	0.0080	0.0028	0.0005	0.0003

Aflatoxin M1	≤0.2 µg/kg	NT	NT	NT	NT	NT	NT

CFU: colony forming units; EU: endotoxin units; NT: not tested.

Table 6. Compositional batch analysis of 2’-FL manufactured with an updated fermentation media without soy peptone: microbial contaminants.

		Batch H	Batch I	Batch J
Parameter	Specification			
Microbial parameters:				
Aerobic plate count	≤1,000 CFU/g	10	10	10
Moulds and Yeasts	≤100 CFU/g	100	100	100
Salmonella	Absent in 100 g	Negative	Negative	Negative
Enterobacteriaceae	Absent in 10 g	Negative	Negative	Negative
Cronobacter spp. (*Enterobacter sakazakii*)	Absent in 100 g	Negative	Negative	Negative
Listeria monocytogenes	Absent in 25 g	Negative	Negative	Negative
Bacillus cereus	≤50 CFU/g	10	10	10
Residual endotoxins	≤10 EU/mg	0.0001563	0.0001563	0.0001563

Aflatoxin M1	≤0.2 µg/kg	≤0.02 (LOQ)	≤0.02 (LOQ)	≤0.02 (LOQ)

CFU: colony forming units; EU: endotoxin units; NT: not tested; LOQ: limit of quantification.

18. The analysis parameters included purity, other carbohydrates, residual proteins, microbial parameters, heavy metals and physicochemical properties (Annex B; p 4 - 9 and Table 3.a-1 of response letter). A purity of 91% to 96% was demonstrated by the analysis. The applicant reports a lack of heavy metals and residual proteins (≤100 mg/kg) and microbial parameters were within limits (Tables 1-6).

19. Other carbohydrate molecules D-lactose (≤3.1% w/w), fucosylgalactose (≤1.0% w/w), difucosyllactose (≤1.4% w/w), L-fucose (≤0.1% w/w), D-glucose and D-galactose (≤0.3% w/w) were detected in low levels. The applicant states that these molecules are natural components or break down products of human breast milk (Annex A; p 33 of dossier).

20. Absence of the genetically modified production organism and no detectable residual DNA in 3 batches of the final product is verified by PCR analysis (Annex A; p 27 – 28 of dossier). Results can be found in Annex C [Annex F].

21. All methods and validation are found in (Annex C [Annex G]). The applicant changed their analytical method for residual proteins from an internal method to a Bradford assay (Annex B; p 4-5 of response letter).

22. The accreditations for the laboratories are found in Annex C [Annex H] – confidential).

Stability

23. The applicant has provided a range of analytical data which characterises and describes the stability of 2’-FL. The applicant reports the results from a 6-month accelerated storage conditions test (40 ± 2°C; 75 ± 5% Relative Humidity) on 5 batches (Annex A; p 28 – 30 and Table 2.c.3.1-1 of dossier). The parameters chosen for measurement and monitoring of stability includes physicochemical and biochemical (purity, carbohydrates and water content). The results show that parameters remained stable within the specifications. The applicant states that the variability in the purity and D-lactose content, was due to malfunctioning

equipment that was subsequently replaced during the course of the study (Annex B; p 19 of response letter).

24. The applicant further demonstrates the stability of parameters of the novel food product under an 18-month normal conditions (25 ± 2°C; 60 ± 5% Relative Humidity) storage test on 1 batch. Microbiological stability is reported with regards to water activity values which remained stable under both accelerated and normal conditions (Annex A; p 31 – 32 of dossier). The applicant states that microbial growth and toxin formation is unlikely due to the low water content and low water activity of the final product (Annex A; p 32 of dossier, and Annex B; p 23 of response letter).

25. Following a request for further information, the applicant revised the proposed shelf life from 3 years to 30 months (2.5 years) to be covered by the data (Annex B; p 19 - 24 of response letter). The applicant further reports results from a 30-month stability test on 1 batch under normal storage conditions (Annex B; p 20 and Table 3.c-1 of response letter) and results from a microbiological stability test at 30 months on 1 batch under normal storage conditions and 6 months on 2 batches under accelerated storage conditions (Annex B; p 24 and Table 3.c-3 of response letter). Parameters remained stable and indicate that no microbial growth or toxin formation was detected.

26. All study reports are found in (Annex C [Annex J]).

Specifications

27. The proposed changes to the specifications of the novel food are shown in Table 7 below (Annex A: p34 – 36, Table 2.d-1 of dossier). Compared with the specifications for authorised 2’-FL synthetically produced or from microbial sources *Escherichia coli* K-12 and *Escherichia coli* BL21 (≥95 % dry matter (DM) ≥ 83 DM and ≥90% DM, respectively), the purity and 2’-FL content of the novel food is lowered to is ≥82% DM.

Table 7. Specifications for 2’-FL.

Description: 2'-FL is a white to off-white powder that is produced by a microbial process. 2'-FL is isolated by spray-drying.

Source:	Genetically modified strain of *Escherichia coli* W
Parameter	Specification
Physicochemical	
Appearance	Powder
Colour	White to off-white
pH	4.0 – 9.0
Composition	
Purity (2’-FL)	≥82 (% dwb)
D-lactose	≤5 w/w %
L-fucose	≤1 w/w %
Fucosylgalactose	≤3 w/w %
Difucosyllactose (difucosyl-d-lactose)	≤3 w/w %
D-glucose and D-galactose	≤1 w/w %
Sum of other carbohydrates (2)	≤10 (% dwb)

Water	≤9.0 w/w %
Ash	≤0.5 w/w %
Residual protein (internal method)	≤10 mg/kg (0.001%)
Residual protein (Bradford assay)	≤100 mg/kg (1)

Heavy metals:

Arsenic	≤0.2 mg/kg
Cadmium	≤0.2 mg/kg
Lead	≤0.2 mg/kg
Mercury	≤0.2 mg/kg
Iron	≤10 mg/kg

Microbial parameters:

Aerobic plate count	≤1,000 CFU/g
Moulds	≤100 CFU/g
Yeasts	≤100 CFU/g

Salmonella	Absent in 100 g
Enterobacteriaceae	Absent in 10 g
Cronobacter spp. *(Enterobacter sakazakii)*	Absent in 100 g
Listeria monocytogenes	Absent in 25 g
Bacillus cereus	≤50 CFU/g
Residual endotoxins	≤10 EU/mg
Aflatoxin M1	≤0.2 µg/kg

2’-FL: 2’-fucosyllactose; CFU: colony forming units; dwb: dry weight basis; EU: endotoxin units. (1) Evaluated using a test limit at 100 ppm; (2) Sum of carbohydrates such as D-lactose, L-fucose, Fucosylgalactose, Difucosyllactose (difucosyl-d-lactose) and D-glucose and D-galactose.

28. The specification parameters for the novel food ingredient were assessed using internationally recognised methods or are otherwise determined using internally developed and validated methods (Annex A; p33 - 36 of dossier and Annex C [Annexes G and H]). The applicant has proposed a new specification of ≤100 mg/kg for residual proteins (Annex B; p 4-5 and Table 3.a-1 of response letter).

History Of Use

29. The applicant reports on the prior authorisations for use of 2’-FL as a novel food in the EU and UK under retained law.

The applicant has conducted a literature search and review on the human biology of background exposure of 2’-FL from human breast milk and discusses likely intake levels based on the history of consumption of human breast milk in infants

(Annex A; p 38 – 39 dossier). The applicant reports the several GRAS notifications in the USA that exist for 2’-FL (Annex A; p 37 of dossier).

30. The applicant summarises the history regarding safety of the production microorganism for laboratory use (Annex A; p 37 of dossier).

Proposed Use and Intake

31. The applicant states that the novel food ingredient is intended to be used in line with the current authorisation for 2 FL by the general population including infants, children and individuals on energy restricted diets for weight reduction.

32. The proposed food uses including a list of current permitted uses and maximum use levels in addition to the newly proposed extension of use in food supplements for infants is presented in Figure 2 below (Annex A; p 40 -41, Table 2.f.2-1 of dossier). The proposed maximum use level in food supplements for infants is 1.2 g/day.

Figure 2. Summary of Authorised Uses for 2’-FL Including the Proposed Extended Condition of Use in Food Supplements for Infants at 1.2 g/day.

Currently Authorised and Proposed Extended Conditions of Use of 2’-FL		
EC Food Category Number	Specified Food Category	Maximum Levels
01	Dairy products and analogues	
01.1	Unflavoured pasteurised and sterilised (including UHT) milk-based product	1.2 g/L

01.2 / 01.3	Unflavoured fermented milk-based products	1.2 g/L beverages 19.2 g/kg products other than beverages
01.4	Flavoured fermented milk-based products including heat-treated products	1.2 g/L beverages 19.2 g/kg products other than beverages
01.8	Dairy analogues, including beverage whiteners	1.2 g/L beverages 12 g/kg products other than beverages 400 g/kg for whitener
07	Bakery wares	
07.2	Cereal bars	12 g/kg
11	Sugars, syrups, honey and table-top sweeteners	
11.4	Table-top sweeteners	200 g/kg
13	Foods intended for particular nutritional uses as defined by Directive 2009/39/EC	

13.1.1	Infant formula as defined in Regulation (EU) No 609/2013	1.2 g/L alone or in combination with up to 0.6 g/L of lacto-N-neotetraose at a ratio of 2:1 in the final product ready for use, marketed as such or reconstituted as instructed by the manufacturer
13.1.2	Follow-on formula as defined in Regulation (EU) No 609/2013	1.2 g/L alone or in combination with up to 0.6 g/L of lacto-N-neotetraose at a ratio of 2:1 in the final product ready for use, marketed as such or reconstituted as instructed by the manufacturer
13.1.3	Processed cereal-based food and baby food for infants and young children as defined in Regulation (EU) No 609/2013	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
13.1.4	Milk-based drinks and similar products intended for young children	1.2 g/L for milk-based drinks and similar products added alone or in combination with up to 0.6 g/L lacto-N-neotetraose, at a ratio of 2:1 in the final product ready for use, marketed as such or reconstituted as instructed by the manufacturer

13.2	Foods 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
13.3	Total diet replacement for weight control as defined in Regulation (EU) No 609/2013	4.8 g/L for drinks 40 g/kg for bars
13.4	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	60 g/kg
14	Beverages	
14.1.4	Flavoured drinks	1.2 g/L
14.1.5	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
17	Food supplements as defined in Directive 2002/46/EC	

17.1 / 17.2	Food supplements as defined in Directive 2002/46/EC	3.0 g/day for general population 1.2 g/day for infants and young children

33. The applicant refers to the estimations of anticipated intakes in infants from previously authorised uses and as evaluated by the EFSA's NDA Panel in 2015. The applicant states that the newly proposed use in food supplements for infants does not alter the dietary exposure assessment or increase the exposure level based on the calculated maximum intake compared to maximum intakes from authorised uses in infants and maximum intakes from breast milk (Annex A; p 41 of dossier).

34. The calculated maximum daily intake of 2'-FL at 1.2 g/day in food supplements for infants on a body weight basis is 240 mg/kg body weight/day (Annex A; p 41 of dossier). The applicant reports that the estimated anticipated maximum intake from breast milk is 1,150 mg/kg body weight/day (Annex A; p 38 - 39 of dossier) and estimated anticipated maximum intakes at the 95th percentile in infants 0 to 6 months, 4 to 6 months, and 7 to 12 months under currently authorised uses are 418, 668, and 641 mg/kg body weight/day, respectively. As such they do not anticipate a concern regarding a lower exposure to the novel food from food supplements for infants in the absence of other exposure sources.

35. The applicant reports that 2'-FL supplements for infants are not intended to be consumed with other sources of 2'-FL on the same day. Therefore, intake and exposure will not be additive or in combination with other sources (Annex A; p 41 - 42 of dossier).

Absorption, Distribution, Metabolism, Excretion (ADME)

36. The ADME does not differ to the ADME of the authorised 2'-FL ingredient. The proposed extension of use presents minor changes to the specification of the final novel food product, of which exposure to related carbohydrate molecules is very low and not expected to be significant or alter the ADME assessment.

37. The applicant reports that the novel food ingredient 2'-FL is structurally and chemically identical to the naturally occurring 2'-FL in human milk and is

considered a non-digestible oligosaccharide. The applicant refers to the EFSA NDA Panel 2015 evaluation and concludes limited digestion in the upper gastrointestinal tract, limited absorption, fermentation by intestinal microbiota and the majority of 2’-FL is excreted in faeces. The ADME of the novel food ingredient is also not expected to differ from the ADME of 2’FL from breast milk and the additional carbohydrate molecules in the final product are naturally occurring components or breakdown products of breast milk (Annex A; p 43 of dossier).

Nutritional Information

39. The applicant states that 2’-FL is not considered nutritionally disadvantageous, as previously evaluated by the EFSA NDA Panel in 2015. 2’-FL is a non-digestible oligosaccharide.

40. The applicant refers to nutrient composition with regards to the low levels of additional carbohydrates in the product composition, in addition to 2’-FL, and that overall exposure to these is expected to be insignificant (Annex A; p 44 of dossier).

Toxicological Information

41. The applicant refers to two publicly available EFSA NDA Panel 2015 evaluations from prior authorisations that report no safety concerns regarding genotoxicity or mutagenicity and a NOAEL of 2000 mg/kg body weight per day. The full study reports are available in Annex C – References – EFSA NDA Panel 2015a, EFSA NDA Panel 2015b.

42. The applicant provides toxicological studies in consideration for the safety of 2’-FL produced using the proposed alternative microbial source. The applicant concludes that the proposed extension of use does not alter the safety of 2’-FL.

43. The applicant conducted a bacterial reverse mutation assay (OECD 471) in accordance with GLP principles and reports that 2’-FL is non-mutagenic at concentrations of up to 5,000 µg/plate, in the absence or presence of metabolic activation (Annex A; p 47 of dossier). The full study report is available in Annex C [Annex K].

44. The applicant conducted an in vivo micronucleus test in mice (OECD 474) in accordance with GLP principles and reports that 2'-FL is non-genotoxic and does not induce chromosomal aberrations in oral doses of up to 2,000 mg 2'-FL/kg

body weight (Annex A; p 47 of dossier). The full study report is available in Annex C [Annex K].

45. The applicant conducted a 90-day oral toxicity study in rats (OECD 408) in accordance with GLP principles and reports a NOAEL of 2,000 mg/kg body weight/day for 2’-FL (Annex A; p 48 of dossier). The full study report is available in Annex C [Annex K] and a summary of statistically significant observations can be found in Annex C [Appendix B.3].

46. The applicant states that there are no safety concerns regarding any antimicrobial susceptibility or antimicrobial production with the proposed genetically modified culture microorganism (Annex A; p 57-59 of dossier). Results are reported from a phenotypic analysis (Annex C [Annex M), genotypic analysis and genome search (Annex C [Annex C and Annex F]) and the applicant concludes absence of acquired antimicrobial resistance or potential for production of antimicrobial compounds.

Allergenicity

47. The applicant states that the purification steps in the production process demonstrate the lack of allergenic potential from any residual proteins ≤1 mg/kg (internal method, Annex C – References – Yamada et al 2004) and ≤100 mg/kg (Bradford assay using a limit test at 100 ppm, Annex B; p 5 – 9 , Table 3.a.1 of response letter) and absence of the production strain or residual DNA in the final product (Annex A; p 60 and p 27 - 28 of dossier).

48. Lactose is added into the production process as a fermentation aid and is also a resulting minor component of the novel ingredient produced by fermentation itself. To exclude the potential for contamination of milk protein allergens, the applicant reports the results of two ELISA tests. Milk proteins were not detected in the final 2’-FL product (Annex A; p 60 of dossier and Annex C [Annex F]). The applicant states that the altered nutrient media following the removal of soy peptone, removes the potential for a soy allergen (Annex A; p 17 – 19 and Table 2.b.1.2-1 of dossier) (Annex C [Annex D]) (Annex B; p 2 of response letter).

49. The applicant conducted a literature search and found no reports of sensitisation, case reports of allergic reactions, or allergenicity studies for 2’-FL (Annex A; p 61 of dossier and Annex C [Annex L])

50. The applicant reports that the allergenic potential of introduced proteins as a result of the genetic modification to the culture microorganism were assessed

using the National Institute of Health Sciences (Japan) Allergen Database for Food Safety (ADFS) (Annex A; p60 of dossier). The applicant concludes that no sequence alerts for potential allergenicity were identified - Annex C [Annex N].

Committee Action Required

- The Committee is asked whether the available data provide a satisfactory basis for evaluating the safety of this novel food ingredient.
- If so, the Committee is asked whether it is content to recommend approval for the amendment to the specifications and conditions of use for 2’-FL as a novel food ingredient to be added to the range of foods specified. A draft Committee Advice Document has been prepared and members comments are sought on the draft.
- If not, the Committee is asked to indicate what additional data would be required.

ACNFP Secretariat

August 2023

Annexes

Annex A – Dossier [Confidential]

Annex B – Request for information with applicant’s responses [Confidential]

Annex C – Supporting documents with annexes, references and appendices [Confidential]

Annex D – Draft Committee Advice Document