Meeting

3-Fucosyllactose (3-FL) Discussion paper

Committee Paper for Discussion - ACNFP/153/03

Advisory Committee For Novel Foods and Processes

Application for Authorisation as a Novel Food for 3-Fucosyllactose (3-FL)

Application number RP1202

Issue

An application has been received under the novel food authorisation process (regulation 2015/2283 as repatriated) for 3-fucosyllactose (3-FL), a source of human identical milk oligosaccharides.

The Committee is asked to advise on whether the available data provides an adequate basis for a risk assessment, and whether the novel food is safe and not nutritionally disadvantageous under the proposed use and use levels.

Background

1. On the 22nd July 2021, the FSA received the submission for 3-FL, a source of human identical milk oligosaccharides from Glycom A/S.

2. The novel food ingredient is made by the fermentation with the production micro-organism, followed by a series of purification, isolation, and concentration steps to generate the purified novel food ingredient. The applicant intends to use the novel food ingredient in conventional foods and beverages, infant formulas, follow-on formula, foods for infants and young children (including processed cereal-based food, baby foods, and milk-based drinks), foods for specific groups (including FSMPs and total diet replacement for weight control), and food

supplements.

3. The application dossier is attached as Annex A and annex to the dossier is attached as Annex B. All contain confidential information.

This application

Identification

4. The novel food ingredient corresponds to the oligosaccharide, 3-fucosyllactose (3-FL). This oligosaccharide is identical in structure to the same molecule that is present in human milk – see Annex B [Annex I – confidential].

5. The novel food ingredient is produced by fermentation with the production micro-organism – see Annex B [Annexes II and III – confidential] – followed by a series of purification, isolation and concentration steps. The final composition is verified by HPLC.

Production Process

6. The applicant states that the manufacturing process is largely comparable to the production processes of previously authorised human identical milk oligosaccharides (*i.e.*, 2'-FL, LNnT, 2'-FL/DFL, LNT, 3'-SL and 6'-SL) – Annex A: p16 dossier. An overview is shown in Table 2.b.2-1 (Annex A: p23 dossier).

Table 1. Overview of the Manufacturing Process for 3-FL

Stage 1		**Upstream processing (USP)**
	Step 1	Media Preparation
	Step 2	Propagation
	Step 3	Seed Fermentation
	Step 4	Fermentation Phases

	Step 4a	Growth (Batch) Phase
	Step 4b	Feeding (Fed Batch) Phase
	Step 4c	Harvest/Storage of Culture Broth
	Step 5	Removal of Microorganism
Stage 2		**Downstream processing (DSP)**
	Step 6	Purification/Concentration 1
	Step 7	Ion Removal
	Step 8	Decolourisation
	Step 9	Purification/Concentration 2
	OP	Crystallisation
	Step 10	Drying
	Step 11	Sampling and Packaging
	Step 12	Quality Control & Batch Release

OP = Optional step

7. Stage 1 of the process involves the conversion of D-lactose and D-glucose to 3-FL by the adapted cellular metabolism of the production microorganism, which uses glucose as an exclusive energy and carbon source and lactose as a substrate for the biosynthesis. At the end of the fermentation, the production micro-organism is removed by filtration (Annex A: p20 – 21 dossier).

8. Stage 2 of the process involves a series of purification and isolation steps to generate the final high-purity ingredient. The crystallisation step with acetic acid is optional, but can be used to minimise certain carbohydrate impurities in the finished product (Annex A: p21 – 22 dossier).

9. The 3-FL ingredient is manufactured in compliance with current Good Manufacturing Practice (cGMP) and the principles of Hazard Analysis Critical Control Point (HACCP) – see Annex A: p22 dossier and Annex B (quality control).

10. The production micro-organism is described in detail (Annex A: p17 – 18 dossier and Annex B [Annex II and III – confidential]).

11. The raw material and processing aids are listed in Table 2.b.1.2-1 (Annex A: p19 – 20 dossier). Specifications are found in Annex D [updated Annex IV – confidential]).

Composition and Specification

12. The applicant has reported analytical data for a number of independent batches of the novel food ingredient concerning carbohydrates (Table 2.c.2.1-1), non-carbohydrate residues (Table 2.c.2.2-1) and microbiological contaminants (Table 2.c.2.3-1). The updated CoA's can be found in Annex B [updated Annex V – confidential].

Table 2. Summary of Batch Results for Carbohydrates in 3'-FL

Parameter	Specification	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	B
Identification by Retention Time	RT of main component corresponds to RT of standard ± 3%	Complies	Complies	Complies	Complies	Complies	Complies	C

Assay (water-free) – Specified saccharides a [%]	≥ 92.0 w/w	96.3	98.5	96.4	95.4	93.8	95.9	9
Assay (water-free) – 3-FL [%]	≥ 87.0 w/w	93.4	97.5	92.1	94.6	93.2	95.8	9
L-Fucose [%]	≤ 1.0 w/w	0.13	0.16	0.39	0.23	0.11	0.03	<
D-Lactose [%]	D-Lactose [%]	≤ 5.0 w/w	2.15	0.29	3.40	0.36	0.30	<
3-Fucosyl-lactulose [%]	≤ 1.5 w/w	0.57	0.56	0.39	0.18	0.12	0.11	<

3-FL = 3-fucosyllactose; RT = retention time.

a Specified saccharides include 3-fucosyllactose, D-lactose, L-fucose and 3-fucosyl-lactulose.

13. The applicant states that L-fucose and D-lactose are recognised constituents of human breast milk. Consumer exposure to L-fucose is expected to be negligible, and not biologically or nutritionally relevant. The quantities of D-lactose present in the novel food ingredient are not considered significant by the applicant (Annex A: p25 dossier).

14. The formation of 3-fucosyl-lactulose occurs by a recognised isomerisation reaction in carbohydrates, and according to the applicant, is a common impurity in other human identical milk oligosaccharides such as 2'-FL, LNnT, 2'-FL/DFL, LNT, 3'-SL and 6'-SL. The level of 3-fucosyl-lactulose is considered to be negligible by the applicant (Annex A: p26 dossier).

15. The applicant has provided further information on the other carbohydrates found in the crystallised 3-FL and the non-crystallised 3-FL. These have qualified and quantified for all batches of the novel food ingredient (Annex D: A-

GDK—2021-5 v1 confidential). The applicant states that these carbohydrates present no safety concerns.

16. The batch results for the non-carbohydrate residues (Table 2.c.2.2-1) shows that the pH of the crystallised 3-FL is lower than the non-crystallised 3-FL. This is due to the presence of acetic acid residues from recrystallisation (Annex A: p26 dossier).

Table 3 Batch Results for Non-carbohydrate Residues in 3-FL

Parameter	**Specification**	**Batch 1**	**Batch 2**	**Batch 3**	**Batch 4**	**Batch 5**	**Batch 6**	**Batch 7**	**Batch 8**
Water (w/w %)	3.2 to 6.0	4.6	5.7	5.7	5.5	3.7	3.8	3.9	3.9
Sulphated ash (w/w %)	≤ 6.0 w/w %	3.60	3.23	3.49	2.41	0.14	0.18	0.04	0.01
pH in 5% solution (20°C)	≤ 0.5 w/w %	< 0.01	< 0.01	< 0.01	0.10	< 0.01	< 0.01	< 0.01	< 0.01
Acetic acid a	≤ 1.0 w/w%	NA	NA	NA	NA	0.5	0.3	0.1	0.1

3-FL = 3-fucosyllactose; NA = not applicable.

a Relevant only for 3-FL crystallised with acetic acid.

17. The applicant reports the absence of the production microorganisms in the bulk ingredient is demonstrated by testing of final batches for bacteria from the *Enterobacteriaceae* family according to internationally-recognised method (ISO 21528-2) – see Table 2.c.2.4-1 below (Annex C: p27 dossier). The updated CoA’s are found in Annex D [updated Annex V – confidential] check

Table 4. Batch Results for Microbiological Analysis of 3-FL

Parameter	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Batch 7	Batch 8
Aerobic mesophilic total plate count	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Enterobacteriaceae	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Salmonella	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Yeasts	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Moulds	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10

3-FL = 3-fucosyllactose; CFU = colony forming units.

18. The applicant states that the quality control steps ensure that manufacturing by-products, impurities and contaminants are monitored. Analysis for the presence of biogenic amines, amino acids and their metabolites did not detect significant levels of these contaminants in the final product (Annex A: p27 dossier).

19. The applicant states that the absence of the production organism in the finished ingredient is also supported by analyses for residual DNA in final production batches. As demonstrated in Table 2.c.2.4-1, the absence of residual DNA from the production organism is confirmed by 3 different quantitative polymerase chain reaction (qPCR) methods (Annex A: p28 dossier).

Table 5. Levels of Residual DNA in Representative Batches of 3-FL

Parameter	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Batch 7	Batch 8

Residual DNA by qPCR (*marc* assay)	< LoQ a	< LoQ	< LoQ	< LoQ	< LoQ	< LoQ	< LoQ	< LoQ
Residual DNA by qPCR (*futA* assay)	< LoQ a	< LoQ	< LoQ	< LoQ	< LoQ	< LoQ	< LoQ	< LoQ
Residual DNA by qPCR (23S assay)	< LoQ a	< LoQ	< LoQ	< LoQ	< LoQ	< LoQ	< LoQ	< LoQ

3-FL = 3-fucosyllactose; DNA = deoxyribonucleic acid; futA = α-1,3-O-fucosyltransferase enzyme; LOQ = limit of quantitation; marc = protein of the Major Facilitator Superfamily; qPCR = quantitative polymerase chain reaction; SD = standard deviation.

a LOQ = 4 µg/kg (parts per billion).

20. The applicant reports the results from the analysis of anions and trace elements which indicate they are not present at any relevant degree. Heavy metals were analysed and found not to be a concern – see Table 2.c.2.4-2 below (Annex A: p29 dossier). The updated CoA's are found in Annex D [updated Annex V – confidential].

Table 6. Levels of Minerals, Trace Elements and Heavy Metals in 8 Batches of 3-FL Produced by Microbial Fermentation

Parameter	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Batch 7	Batch 8
Arsenic (mg/kg)	< 0.1	< 0.1	< 0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1

Cadmium (mg/kg)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Mercury (mg/kg)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Copper (mg/kg)	0.2	0.3	0.5	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Lead (mg/kg)	< 0.01	< 0.01	< 0.01	< 0.05	< 0.01	< 0.01	< 0.01	< 0.01
Manganese (mg/kg)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Nickel (mg/kg)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Ammonium (w/w%)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Calcium (w/w%)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Chloride (w/w%)	0.010	0.001	0.001	0.012	0.009	0.005	< 0.004	< 0.004
Iron (mg/kg)	0.5	< 0.1	< 0.5	< 10	0.6	< 2	< 0.5	< 0.5
Magnesium (w/w%)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.0005
Phosphate (w/w%)	< 0.0005	< 0.0005	< 0.0005	< 0.0005	< 0.0006	< 0.0005	< 0.0005	< 0.0006

Potassium (w/w%)	< 0.001	0.001	< 0.001	< 0.001	0.002	0.001	< 0.001	< 0.001
Sodium (w/w%)	0.010	0.006	0.007	0.011	0.003	0.001	< 0.001	< 0.001
Sulphate (w/w%)	< 0.0024	0.0004	< 0.004	0.0239	< 0.0051	< 0.0043	< 0.004	< 0.005
Zinc (mg/kg)	0.4	0.1	< 0.1	< 0.5	< 0.2	< 0.1	< 0.1	< 0.1
Selenium (mg/kg)	< 0.05	< 0.1	< 0.1	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Molybdenum (mg/kg)	< 0.1	< 0.1	< 0.1	< 0.2	< 0.1	< 0.1	< 0.1	< 0.1

3-FL = 3-fucosyllactose

21. The applicant reports the results from the analysis of microbial endotoxins and proteins which indicate that the levels detected were not a safety concern. The permitted level of endotoxins are specified by the applicant at ≤10 endotoxin units (EU)/mg (Annex A: p23 dossier). See Table 2.c.2.4-3 below (Annex A: p30 dossier). The updated CoA's are found in Annex D [updated Annex V] – confidential.

Table 7. Batch Results for Microbial Endotoxins and Residual Proteins in 3-FL

Parameter	**Batch 1**	**Batch 2**	**Batch 3**	**Batch 4**	**Batch 5**	**Batch 6**	**Batch 7**	**Batch 8**

Residual endotoxins [E.U./mg]	0.0025	0.0066	0.0122	0.0016	< 0.00025	0.0498	0.011	0.0007
Residual protein by Bradford assay [%]	< 0.0017	< 0.0017	< 0.0017	< 0.001	< 0.0017	< 0.0017	0.004	< 0.0017

3-FL = 3-fucosyllactose; E.U = endotoxin units

22. The methods and validation are provided in Annex B [Annex VI – confidential].

23. The accreditation for the laboratories are found in Annex B [Appendix B2].

Stability

24. The applicant reports the results from a 5-year real-time stability study in bulk at 25oC and 60% relative humidity in two batches of novel food ingredient (one crystallised 3’-FL and one non-crystallised 3’-F). Currently, only results are reported for a period of 18 months are available (Annex A: Tables 2.c.3.1-1 and 2.c.3.1-2, p30 – 31 dossier) which show no significant degradation of 3’-FL. The CoA’s can be found in Annex B [updated Annex VII – confidential].

25. The applicant has reported two further samples of novel food ingredient at ambient temperature and humidity – one batch covering 22 months and a second batch covering 24 months. The CoA’s can be found in Annex D [A-GDK-2022-007 and 008].

26. The applicant reports the interim results from a 2-year accelerated stability study in bulk at 40oC and 70% relative humidity in two batches of novel food ingredient (one crystallised 3-FL and one non-crystallised 3-F). Results provided for the first 18 months only (Annex A: Tables 2.c.3.1-3 and 2.c.3.1-4, p32 – 33 dossier) show no appreciable degradation of 3-FL or changes in the impurity profile. The CoA’s can be found in Annex D [updated Annex VII – confidential].

27. The applicant reports the results from a stress and forced stability study under a range of conditions and identified four potential degradation pathways (Annex A: p33 – 34 dossier). The pH of the solution was found to influence the

formation of degradation products (see Figure 2.c.3.1-1). The results of these studies are reported in Annex D [updated Annex VII – confidential].

28. The applicant reports the results from a 3-year stability study in powdered infant formula at various temperatures. Results were reported covering a period of 12 months (Annex A: Table 2.c.3.2-1, p34 – 35 dossier). The results indicate that novel food ingredient shows good stability. The CoA's can be found in Annex D [updated Annex VII – confidential].

29. The applicant highlights results that show 3-FL is reported stable in UHT milk, whole milk, and yoghurt. Further, the applicant states that based on its structure, the stability of LNFP-I is anticipated to be highly similar to 2'-FL, LNT, and LNnT which have been approved as novel food ingredients in the EU and UK under retained law (Annex A: p35 dossier).

Specification

30. The specification parameters for the novel food ingredient were assessed using internationally recognised methods or are otherwise determined using internally developed and validated methods – see Table 2.d-1 (Annex A: p36 dossier).

Table 8. Product Regulatory Specifications for 3-FL

Parameter	Specification	Method
Appearance	Powder, agglomerates, powder with agglomerates	ISO 6658
Colour	White, white to off-white, off-white	ISO 6658
Identification by Retention Time	RT of main components correspond to RT of standards ± 3%	Glycom method HPLC-13-002

Assay (water-free) – Specified saccharidesa	≥ 92.0 w/w %	Glycom method HPLC-13-001, HPLC-13-002, HPAEC-HMO-017
Assay (water-free) – 3'-FL	≥ 87.0 w/w %	Glycom method HPLC-13-002
L-Fucose	≤ 1.0 w/w %	Glycom method HPAEC-HMO-017
D-Lactose	≤ 5.0 w/w %	Glycom method HPAEC-HMO-017
3-Fucosyllactose	≤ 1.5 w/w %	Glycom method HPAEC-HMO-017
Sum of other carbohydrates	≤ 5.0 w/w %	Glycom method HPAEC-HMO-017
pH in 5% solution (20°C)	3.2–6.0	Ph. Eur. 2.2.3
Water	≤ 6.0 w/w %	Glycom method KF-001
Ash, sulphated	≤ 0.5 w/w %	Ph. Eur. 2.4.14
Acetic acid	≤ 1.0 w/w %	
Residual protein by Bradford assay	≤ 0.01 w/w %	Glycom method UV-001
Residual endotoxins	≤ 10 EU/mg	Ph. Eur 2.6.14 (LAL kinetic chromogenic assay)

Lead	≤ 0.1 mg/kg	EN 13805; EPA-6020A
Aerobic mesophilic total plate count	≤ 1,000 CFU/g	ISO 4833-1 or ISO-4833-2
Enterobacteriaceae	≤ 10 CFU/g	ISO 21528-2 or NMKL 144
Salmonella	Absent in 25 g	ISO 6579 or AFNOR BRD 07/11-12/05
Yeasts	≤ 100 CFU/g	ISO 21527-2
Moulds	≤ 100 CFU/g	ISO 21527-2

CFU = colony forming units; EPA = Environmental Protection Agency; E.U. = endotoxin units; HPAEC = high-performance anion exchange chromatography; HPLC = high-performance liquid chromatography; ISO = International Organization for Standardization; KF = Karl-Fischer; Ph. Eur. = European Pharmacopoeia; RT = retention time; UV = ultraviolet.

a Specified saccharides include 3-fucosyllactose, D-lactose, L-fucose and 3-fucosyl-lactulose.

b Relevant only for crystallised 3-FL.

History of Use

31. Statement that to the applicant’s knowledge 3-FL has not been “used” as a food ingredient anywhere in the world to date, however, the production organism has been used to manufacture 2'-FL, LNnT, 2'-FL/DFL, LNT, 3'-SL and 6'-SL which have all been authorised as novel foods in the EU and UK under retained law (Annex C: p37 dossier).

32. The applicant provides a review on the human biology of human breast milk with reference to 3-FL and discusses the likely intake levels of this oligosaccharide based on the consumption of human breast milk (Annex A: p37 – 40 dossier).

Proposed Use and Intake

33. The applicant states that the novel food ingredient is intended to be used by infants, children and adults, including pregnant and lactating women (Annex A: p41 dossier).

34. The proposed food uses and maximum use levels are listed in Table 2.f.2-1 below (Annex A: p41 – 42 dossier).

Table 9. Proposed Food Uses and Use Levels for 3-FL

EU Food Category Number a	Food Category Name	Proposed Maximum Use Level (expressed as LNFP-I)
1	**Dairy Products and Analogues**	
1.1	Unflavoured pasteurised and unflavoured sterilised (including UHT) milk	2.0 g/L
1.2/1.3	Unflavoured fermented milk-based products	2.0 g/L beverages
1.4	Flavoured fermented milk-based products including heat-treated products	4.0 g/kg products other than beverages
7	**Bakery wares**	2.0 g/L beverages

7.2	Fine bakery wares. Cereal bars only	12.0 g/kg products other than beverages
13	**Foods for Special Groups (FSG)**	
13.1	**Foods for infants and young children**	25.0 g/kg
13.1.1	Infant formula as defined in Regulation (EU) No 609/2013	2.0 g/L 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	2.0 g/L 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	2.0 g/L in the final product ready for use, marketed as such or reconstituted as instructed by the manufacturer 12.0 g/kg for products other than beverages
13.1.4	Milk-based drinks and similar products intended for young children	1.2 g/L in the final product ready for use, marketed as such or reconstituted as instructed by the manufacturer 10 g/kg for products other than beverages

13.2	**Foods for special medical purposes as defined in Regulation (EU) No 609/2013**	
13.2	Foods for special medical purposes as defined in Regulation (EU) No 609/2013	On case-by-case basis
13.3	**Total diet replacement for weight control as defined in Regulation (EU) No 609/2013**	
13.3.3	Total diet replacement for weight control as defined in Regulation (EU) No 609/2013	2.0 g/L 25.0 g/kg for products other than beverages
14	**Beverages**	
14.1.4	Flavoured drinks (excluding cola-type drinks)	1.25 g/L

3'-FL = 2'-fucosyllactose; EU = European Union; UHT = ultra-high temperature.

a Numbering in the reference to the Guidance document describing the food categories in Part E of Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives and the Union List Entry for 2'-FL, See Part E, Annex II Consolidated version of: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02008R1333-20200325&from=EN.

35. The applicant states that the intake of 3-FL in infants up to the age of 16 weeks at P95 is estimated to be 520 mg/kg body weight/day, equivalent to 3.48 g/day for a 6.7kg infant using EFSA draft formula intake levels (Annex A: p42 dossier).

36. The applicant determined the intake for the novel food ingredient from all conventional foods and beverages (see Annex A: Table 2.f.3.2.2-1, p44 dossier) using the EFSA Comprehensive Database (Annex A: p43 – 44 dossier). Detailed analysis can be found in Annex B [Annex X – confidential].

37. The applicant reports that on a body weight basis, the highest intakes of 3-FL were observed in infants and toddlers, with mean intakes of up to 196 and 119 mg/kg body weight/day, respectively, and high-level intakes at up to 499 and 231 mg/kg body weight/day, respectively. A summary of this analysis can be found in Table 2.f.3.2.3-1 for all population groups (Annex A: p45 dossier).

Table 10. Estimated Daily Intake of 3-FL from Proposed Food Uses Based on Summary Statistics from the EFSA Comprehensive Database (EFSA, 2020a) in Different Population Groups

Population Group	No. of surveys*	Mean Intakes of 3-FL (mg/day)	High Level Intakes of 3-FL (mg/day)	Mean Intakes of 3-FL (mg/kg bw/day)	High Level Intakes of 3-FL (mg/kg bw/day)
Infants (≤ 11 months)	13 (11)	377 to 1,148	1,352 to 3,028	44 to 196	155 to 499
Toddlers (12 to 35 months)	20 (17)	473 to 1,189	983 to 2,373	38 to 119	92 to 231
Other children (3 to 9 years)	30 (30)	395 to 1,262	883 to 2,002	13 to 66	34 to 116

Adolescents (10 to 17 years)	30 (29)	185 to 1,326	716 to 2,381	3 to 25	16 to 49
Adults (18 to 64 years)	35 (35)	157 to 967	697 to 2,176	2 to 13	11 to 29
Pregnant and lactating women	7 (7)	326 to 907	906 to 1,789	5 to 13	12 to 28
Elderly (65 to 74 years)	25 (23)	151 to 777	605 to 2,195	2 to 10	10 to 27

3-FL = 3-fucosyllactose; bw = body weight; EFSA = European Food Safety Authority.

a Number of surveys in which individuals within the population group were identified to be consumers of the food groups of interest. Number of surveys with a statistically reliable number of consumers (used to calculate high level intakes) are presented in parentheses.

b Results are not presented that were not statistically reliable (n < 60).

38. The applicant states that the wide range of intake values at P95 for infants under the age of 11 months represent the variability in the national survey data and the assumption that the proposed foods will be consumed at the maximum proposed intake levels, which is deemed extremely unlikely (Annex A: p45 dossier).

39. The applicant concludes that the intake of 3-FL does not exceed the levels found in human breast milk for which the there is a history of safe use – see Table 2.f.3.3-1 (Annex A: p46 dossier and Annex B [Annex IX – confidential]).

Table 11. Highest Intakes of 3-FL from Breast Milk vs. Highest Level of Exposure from Proposed Uses

Dietary Source	Estimated Daily Intake for 3-FL (mg/kg bw/day)
Mean Intake of Human Milk (800 ml) for 6.7 kg infant	
Highest Mean (based on 3.43 g/L a)	410
Highest 95% CL (based on 4.72 g/L a)	563
Highest Mean Consumption from Proposed Uses b	195
Mean Intake of Human Milk (1200 ml) for 6.7 kg infant	
Highest Mean (based on 3.43 g/L b)	614
Highest 95% CL (based on 4.72 g/L b)	845
Highest 95% Percentile Consumption from Proposed Uses b	499

3-FL = 3-fucosyllactose; bw = body weight; CL = confidence limit

a See Section 2.e.3.

b Estimated intake for all infants.

40. The applicant states that the use of the novel food ingredient in food supplements is expected to give estimated intake levels of up to 173 mg/kg bw/day for children 3 – 9 years old, 168 mg/kg bw/day for toddlers and 299 mg/kg

bw/day for infants under the age of 11 months. All other age groups ranged from 53 mg/kg bw/day in the elderly to 92 mg/kg bw/day in adolescents (Annex A: p46 dossier).

41. The applicant concludes that consumption of the novel food would lead to significantly lower intake levels for 3-FL compared to consumers of breast milk – see Table 2.f.3.4-2 below (Annex A: p47 dossier and Annex B [Annex IX – confidential]).

Table 12. Highest Intakes of 3-FL from Breast Milk vs. Highest Level of Exposure from Food Supplements Consumed by 6.7kg infant

Highest mean intake based on 800 ml breast milk	**Highest 95% CL intake based on 800 ml breast milk**	**Highest mean intake based on 1200ml breast milk**	**Highest 95% CL intake based on 1200ml breast milk**	**Highest intake at 95% percentile from proposed use as a food supplement at 2 g/day**
410 mg/kg bw/day	563 mg/kg bw/day	614 mg/kg bw/day	845 mg/kg bw/day	299 mg/kg bw/day

3-FL = 3-fucosyllactose; bw = body weight; CL = confidence limit

Highest Mean (based on 3.43 g/L)

Highest 95% CL (based on 4.72 g/L)

42. The applicant proposes a series of statements for the labelling of products containing the novel food ingredient to provide assurance that consumer should avoid excessive intake from consuming food products and food supplements together. This is reported to be consistent with the approach mandated for 2’-FL (2’-fucosyllactose) on Table 1 of the Union list of novel foods under Commission Regulation (EC) 2017/2340 (Annex A: p47 dossier).

Absorption, Distribution, Metabolism and Excretion (ADME)

43. The applicant states that 3-FL in the novel food ingredient is structurally identical to their naturally occurring counterparts in human milk. There is no significant digestion in the upper gastrointestinal tract, limited oral absorption and a small proportion is excreted unchanged. The absorption of the novel food ingredient is not expected to be different from absorption from breast milk and is not expected to pose a safety concern (Annex A: p49 dossier).

Nutritional Information

44. The applicant refers to the EFSA Scientific Opinion on the Essential composition of infant and follow-on formulae which states: “Human milk oligosaccharides are not considered in the estimation of the energy content of the milk.”.

45. The applicant proposes labelling the novel food ingredient as *“non-digestible (non-glycaemic) oligosaccharide”*.

Toxicological Information

46. The applicant reports that results from a bacterial reverse mutation assay (OECD TG 471) show that 3-FL is non-mutagenic at concentrations up to 5,000 µg/plate, in the absence or presence of metabolic activation (Annex A: p53 dossier). The full study report is available in Annex A – References – Gilby, 2021a [unpublished] – confidential.

47. The applicant reports that results from an *in vitro* mammalian cell micronucleus test (OECD TG 487) using 3-FL is neither clastogenic nor aneugenic up to a dose of 2000 µg/ml. (Annex A: p53 – 54 dossier). The full study report is available in Annex A – References – Gilby, 2021b [unpublished] – confidential.

48. The applicant reports a sub-chronic (90-day) oral toxicity study in rats utilising the OECD guideline 408 conducted to GLP principles. The 3-FL was reported to be well tolerated at doses up to 4,000 mg/kg body weight/day (Annex A: p55 – 57 dossier). The full study report is available in Annex A – References – Stannard, 2021b [unpublished] – confidential.

Allergenicity

49. The applicant states that the high purification steps involved in the manufacture are proven to remove protein (*i.e.*, potential allergen) to a level of < 0.01% w/w (Annex A: p61 dossier).

50. The applicant reports the allergenic potential of introduced proteins as a result of the genetic modification of the micro-organism were assessed using the Allergen Online tool (ver. 21) of the University of Nebraska. No sequence alerts for potential allergenicity were identified – Annex B [Annex II – confidential] for the full amino acid sequences.

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 of the novel food as an ingredient to be added to the range of foods specified.
- If not, the Committee is asked to indicate what additional data would be required.

Annexes (Confidential)

ACNFP-153-03-Annex A – Dossier and References [Confidential]

ACNFP-153-03-Annex B – Annexes [Confidential]

ACNFP-153-03-Annex C – Request For Information

ACNFP-153-03-Annex D – Applicant’s Response to Request For Information