Safety Assessment: Outcome of the assessment of 3-fucosyllactose (3-FL) as a novel food

Maes o ddiddordeb ymchwil: Research projects
Statws y prosiect: Wedi'i gwblhau
Cod prosiect: RP1202
Cynhaliwyd gan: Food Standards Agency (FSA) and Food Standards Scotland (FSS)
Dyddiad cyhoeddi: 14 Awst 2023
DOI: https://doi.org/10.46756/sci.fsa.crr177
PDF
Gweld Safety Assessment: Outcome of the assessment of 3-fucosyllactose (3-FL) as a novel food as PDF(Open in a new window) (588.46 KB)

Summary

The novel food is 3-FL which is intended to be used as a source of human identical milk oligosaccharides. 3-FL is manufactured by microbial fermentation using a genetically modified strain of *Escherichia coli* K-12, and then refined to yield the purified novel food.

This new application is seeking to use the novel food within the food following categories: dairy products and analogues, bakery wares, foods for special groups, beverages, and also as a food supplement. Food supplements are not intended to be used if other foods with added 3-FL or breast milk are consumed the same day.

To support the FSA and FSS in their evaluation of the application, the Advisory Committee on Novel Foods and Processes (ACNFP) were asked to review the safety dossier and supplementary information provided by the applicant. The Committee concluded that the applicant had provided sufficient information to assure the novel food, 3-FL, was safe under the proposed conditions of use. The anticipated intake levels and the proposed use in foods and food supplements was not considered to be nutritionally disadvantageous and does not mislead consumers.

The views of the ACNFP have been taken into account in the regulatory assessment which represents the opinion of the FSA and FSS.

Introduction

1. FSA and FSS have undertaken a risk assessment for 3-FL under the novel foods legislation, Regulation (EU) 2015/2283, as retained in UK law. To support the risk assessment, the ACNFP provided the advice outlined in this opinion to the FSA and FSS.

2. The evaluation by the ACNFP assessed the food safety risks of the novel food and its production, in line with Article 7 of Commission Implementing Regulation (EU) 2017/2469, as

retained in UK law. The regulatory framework and the technical guidance put in place by the European Food Safety Agency (EFSA) for full novel food applications is retained as the basis and structure for the assessment (EFSA NDA Panel, 2021).

3. With thanks to the members of the ACNFP during the course of the assessment who were; Dr Camilla Alexander White, Dr Anton Alldrick, Alison Austin, Dr Mark Berry, Professor Dimitris Charalampopoulos, Professor Susan Duthie, Professor Susan Fairweather-Tait, Professor Paul Frazer, Dr Hamid Ghoddusi, Professor Andy Greenfield, Professor Wendy Harwood, Professor Huw Jones, Dr Ray Kemp, Dr Elizabeth Lund, Nichola Lund, Dr Rohini Manuel, Emeritus Professor Harry McArdle, Rebecca McKenzie, Professor Clare Mills, Dr Lesley Stanley, Professor Hans Verhagen, Dr Maureen Wakefield and Professor Bruce Whitelaw.

4. Following the review by the ACNFP at their meeting in June 2022, further clarification was requested concerning the production process of 3-FL, in order to address information gaps in the dossier. The final recommendations from the Committee were presented at the 157th meeting, allowing the FSA and FSS to complete the risk assessment.

5. The views of the ACNFP have been taken into account in this safety assessment which represents the opinion of the FSA and FSS on 3-FL.

Assessment

Identity of novel food

6. The novel food is 3-fucosyllactose (3-FL) which is a purified white to off-white powder or agglomerate containing ? 90% 3-FL by dry weight. Due to the presence of other saccharides present in smaller quantities, the total saccharide content of the novel food is ? 92% by dry weight.

7. 3-FL is a trisaccharide consisting of D-galactose, D-glucose and L-fucose (see Diagram 1). 3-FL is characterised by the chemical formula: $C_{18}H_{32}O_{15}$; molecular mass: 488.44 g/mol; CAS number: 41312-47-4; IUPAC name: ?-D-Galactopyranosyl-(1?4)-[?-L-fucopyranosyl-(1?3)]-D-glucose.

Diagram 1: The molecular structure of 3-FL

8. Confirmation that the 3-FL in the novel food, is equivalent to 3-FL found in human breast milk was provided by comparative nuclear magnetic resonance (NMR) spectroscopy: ^{1}H-NMR, ^{13}C-NMR and 2D Nuclear Overhauser Effect spectroscopy (NOESY) analysis.

9. Information to support this characterisation was provided for eight batches of the novel food: four batches of crystallised 3-FL and four batches of non-crystallised 3-FL.

Production Process

10. The production microorganism used to manufacture the novel food is a genetically modified derivative of *E. coli* K-12 DH1 MDO that functions as a processing aid as defined in Article 3(2)(b) of Regulation (EC) No.1333/2008 on food additives, as retained in UK law. A novel food produced by a GMO does not fall under the remit of the GMO legislation, Regulation (EC) No 1829/2003, as retained in UK law, or Regulation (EC) No 1830/2003, as retained in UK law, when the production microorganism, *E. coli* K-12, is removed during the manufacturing process and therefore no recombinant DNA remains. This has been confirmed in the compositional analysis as detailed below.

11. The novel food is classified as category 1 under the EFSA GMO guidance: chemically defined purified compounds and their mixtures in which both genetically modified microorganisms (GMMs) and newly introduced genes have been removed, under EFSA guidance which categorises GMMs and their products for risk assessment purposes (EFSA GMO Panel, 2011), which the FSA have retained for the purposes of technical review.

12. The microorganism, *E. coli* K-12, has not been adopted into the Qualified Presumption of Safety list; however, other human identical milk oligosaccharides manufactured using the same strain of microorganism are present in the List of Novel Foods, Regulation (EU) 2017/2470, as retained in UK law. This includes 2'-FL, LNnT (Lacto-N-neotetraose), 2'-FL/DFL (difucosyllactose), LNT (Lacto-N-tetraose), 3'-SL (3’-sialyllactose sodium salt) and 6'-SL (6’-sialyllactose sodium salt).

13. Information on the hazard identification, hazard characterisation, and exposure assessment for the genetically modified derivative of *E. coli* K-12 DH1 MDO was provided in line with EFSA guidance (EFSA GMO Panel, 2011).

14. Stage 1 of the production process involves the conversion of D-lactose and D-glucose (D-sucrose or glycerol are alternatives to D-glucose) to 3-FL by the adapted cellular metabolism of the production microorganism. Glucose acts as an exclusive energy and carbon source, and lactose as a substrate for the biosynthesis of 3-FL. The 3-FL is released from the *E. coli* K-12 into the fermentation broth. At the end of the fermentation, the *E. coli* K-12 is removed by filtration (see Diagram 2).

Diagram 2: Overview of the Manufacturing Process for 3'-FL

Stage 1	**Upstream Processing (USP)**
Steps	1 Media Preparation 2 Propagation 3 Seed Fermentation 4 Fermentation Phases 5 Removal of microorganism*

Stage 2	**Downstream Processing (DSP)**
Ste[s	6 Purification/Concentration 1* 7a Ion Removal 8a Decolourisation 9 Purification/Concentration 2* OP Crystallisation 10 Drying 11 Sampling and Packaging 12 Quality Control & Batch Release

OP = optional stage
a The order of steps is interchangeable.
* After the marked steps additional sterile filtration (microfiltration) is performed to maintain low microbial load during all times of downstream processing and to ensure high microbial quality of the final ingredient. These steps are further reassurance of absence of the production microorganism in final ingredient.

15. Stage 2 of the production process involves a series of purification and isolation steps to generate the final high-purity ingredient (see Diagram 2). The crystallisation step with acetic acid is optional, but can be used to minimise certain carbohydrate impurities in the finished product.

16. The certificates of analysis for the raw materials and the processing aids used in the manufacture of the novel food are provided. D-Glucose, D-lactose, D-sucrose and glycerol are food grade. The ‘glucose’ contains ? 99.5% glucose. The ‘lactose’ contains ? 99% lactose with lactulose (0.05% maximum) and protein (0.2% maximum). The ‘sucrose’ contains ? 71% sucrose with inverted sugar (0.5% maximum). The ‘glycerol’ contains ? 98% glycerol with moisture content ? 5%.

17. The novel food is produced in compliance with current Good Manufacturing Practice (cGMP) and the principles of Hazard Analysis Critical Control Point (HACCP). Manufacturing by-products, impurities and contaminants from the fermentation broth are monitored. Analysis from manufacturing batches did not detect significant levels of biogenic amines or amino acids and their metabolites in the final product.

18. The absence of bacteria from the *Enterobacteriaceae* family (ISO 21528-1:2004, MSZ ISO 21528-2:2004) and residual bacterial DNA (futC assay; marc assay; 23S assay: LOQ = 4 µg/kg for all assays) confirms the genetically modified *E. coli* K-12 is not present in the novel food.

19. Analytical data from eight batches of novel food, four batches of non-crystallised 3-FL and four batches of crystallised 3-FL, confirmed the presence of very low levels of microbial endotoxins and proteins (Table 1).

Table 1: Batch results for microbial endotoxins and residual proteins in the novel food

Parameter	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Batch 7	Batch 8
Residual endotoxins (EU/mg)	0.0066	0.0122	0.0016	< 0.0003	0.0498	0.0110	0.0007	0.0077
Residual protein by Bradford assay (w/w %)	< 0.0017	< 0.0017	< 0.0017	< 0.001	< 0.0017	< 0.0017	0.00405	< 0.0017
Aflatoxin M1 (µg/kg)	< 0.02	< 0.02	< 0.02	NT	< 0.02	< 0.02	< 0.02	NT

Batches 1 – 4 Non-crystallised 3-FL; Batches 5 – 8 Crystallised 3-FL
NT = not tested

20. The production process has characterised the potential hazards and detailed the corresponding control measures sufficiently.

Compositional Information

21. Results from eight independent batches of the novel food, four batches of non-crystallised 3-FL and four batches of crystallised 3-FL, demonstrated that the carbohydrate content consistently meets the proposed specification levels (Table 2).

Table 2: The carbohydrate content of the novel food

Parameter	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Batch 7	Batch 8
Identification by Retention Time	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass

Parameter	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Batch 7	Batch 8
Assay (water-free) – Specified saccharides a [%]	96.2	98.5	96.3	95.4	93.8	95.9	97.4	96.5
Assay (water-free) – 3-FL [%]	93.36	97.45	92.11	94.63	93.23	95.77	97.44	96.51
L-Fucose [%]	0.13	0.16	0.39	0.23	0.11	0.03	< 0.03	< 0.03
D-Lactose [%]	2.15	0.29	3.40	0.36	0.30	< 0.03	< 0.03	< 0.03
3-Fucosyl-lactulose [%]	0.57	0.56	0.39	0.18	0.12	0.11	< 0.03	< 0.03
Sum of other carbohydrates [%]	0.46	0.73	0.82	1.76	2.70	1.52	0.75	0.98
pH in 5% solution (20°C)	4.6	5.7	5.7	5.5	3.7	3.8	3.9	3.9
Water	3.60	3.23	3.49	2.41	0.14	0.18	0.04	0.01
Sulphated ash	< 0.01	< 0.01	< 0.01	0.10	< 0.01	< 0.01	< 0.01	< 0.01
Acetic acid b	NA	NA	NA	NA	0.5	0.3	0.1	0.1

Batches 1 – 4 Non-crystallised 3-FL; Batches 5 – 8 Crystallised 3-FL

Specification Limits: RT of main component corresponds to RT of standard ± 3%; Specified saccharides ? 92.0% w/w; 3-FL ? 90.0% w/w; L-fucose ? 1.0% w/w; D-lactose ? 5.0% w/w; 3-fucosyl-lactulose ? 1.5% w/w; sum of other carbohydrates ? 5.0% w/w;;pH in 5% solution (20°C) – 3.2 to 7.0; water ? 6.0% w/w; sulphated ash ? 5.0% w/w; acetic acid ? 1.0% w/w
a Specified saccharides include 3-fucosyllactose, D-lactose, L-fucose and 3-fucosyl-lactulose.
b Relevant only for 3-FL crystallised with acetic acid.

22. 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 3-fucosyl-lactulose is formed by a recognised isomerisation reaction in carbohydrates, but the level is considered to be negligible. This particular oligosaccharide is a recognised impurity in other human identical milk oligosaccharides such as 2'-FL, LNnT, 2'-FL/DFL, LNT, 3'-SL and 6'-SL, which are present in the Union List of Novel Foods, Regulation (EU) 2017/2470, as retained in UK law.

23. The batch results for the non-carbohydrate residues showed 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 and not expected to be a cause for concern under the proposed conditions of use.

24. The fermentation medium contains minerals and trace elements, with trace metals functioning as co-factors for different enzymes. The results from eight independent batches of the novel food, four batches of non-crystallised 3-FL and four batches of 3-FL of the novel food, demonstrated the filtration and purification steps remove these minerals and trace elements to very low levels (Table 3). Analytical data also confirmed the levels of heavy metals were either below the limit of detection or present in very low levels.

Table 3: The content of heavy metals, minerals and trace elements in the novel food

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

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

Batches 1 – 4 Non-crystallised 3-FL; Batches 5 – 8 Crystallised 3-FL
Manganese (< 0.1 mg/kg); nickel (< 0.1 mg/kg); ammonium (< 0.005 w/w%); calcium (< 0.001 w/w%); magnesium (< 0.001 w/w%); phosphorous (< 0.0005 w/w%); molybdenum (< 0.2 mg/kg); selenium (< 0.1 mg/kg) – reported values that were below the limit of detection for all batches of the novel food

25. Analytical data concerning the microbiological content from eight independent batches of the novel food, four batches of non-crystallised 3-FL and four batches of 3-FL, of the novel food were reported (Table 4). The results confirm that the novel food consistently meets the proposed specification levels.

Table 4: The microbiological analysis of the novel food

Parameter	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Batch 7	Batch 8
Aerobic mesophilic total plate count (? 1,000 CFU/g)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Enterobacteriaceae (absent in 10g)	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Salmonella (absent in 25 g)	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Yeasts (? 100 CFU/g)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Moulds (? 100 CFU/g)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Bacillus cereus (? 50 cfu/g)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Listeria monocytogenes (absent in 25g)	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent

Parameter	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Batch 7	Batch 8
Cronobacter spp. (absent in 10g)	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent

Batches 1 – 4 Non-crystallised 3-FL; Batches 5 – 8 Crystallised 3-FL

26. Certification was provided to demonstrate that the contract laboratories were accredited to perform these analytical studies. Where in-house analysis was used, full methodology and supporting validation documentation was provided.

27. The data presented indicate the novel food can be consistently produced within the proposed specification.

Stability

28. The stability of the novel food was assessed under real-time conditions (25^{o}C and 60% relative humidity) in two batches, one crystallised and one non-crystallised over a period of eighteen months. Results showed that the novel food meets the specification criteria for saccharide content and microbiological quality over this time period. Data for a further two batches of the novel food under ambient temperature and humidity, one batch covering 22 months and the second batch covering 24 months, were provided following the same parameters. Both batches met the specification limits.

29. The stability of the novel food was assessed under accelerated conditions in two batches of non-crystallised 3-FL over a period of 18 months. Results confirmed that the novel food meets the specification criteria for saccharide content and microbiological quality over this time period.

30. Results from a stability study of powdered infant formula containing the novel food under various environmental conditions (5^{o}C; 25^{o}C and 60% RH; 30^{o}C and 65% RH; 40^{o}C and 70% RH) over a period of 12 months confirmed, that the 3-FL content and microbiological quality of the novel food, is stable over this time period. Additional supporting evidence from a published study by Christensen et al. (2020) reported that 3-FL is stable in UHT milk (3 months), whole milk, and yoghurt (up to 36 days). Based on its structure, the stability of 3-FL is anticipated to be similar to those of 2'-FL, LNT, and LNnT, which have been approved as food ingredients in the List of Novel Foods, Regulation (EU) 2017/2470, as retained in UK law.

31. The data provided supports the stability of the novel food for a time period of at least 12 months.

Specification

32. The specification parameters reported in Table 5 were assessed using internationally recognised methods or determined using internally developed and validated methods.

Table 5: Specification of the novel food

Description
3-Fucosyllactose is a purified carbohydrate powder or agglomerate obtained from microbial fermentation with a genetically modified strain of *Escherichia coli* K-12 DH1 containing at least 90% of 3-fucosyllactose of dry matter.

Parameter	Specification	Method

Appearance	Powder, agglomerates, powder with agglomerates	ISO 6658
Colour	White, white to off-white, off-white	ISO 6658
Assay (water-free) – Specified saccharides a	? 92.0 w/w %	Glycom method HPLC-402-4C4-001, HPAEC-HMO-021, and HPLC-3FL-003
Assay (water-free) – 3'-FL	? 90.0 w/w %	Glycom method HPLC-402-4C4-001
L-Fucose	? 1.0 w/w %	Glycom method HPLC-3FL-002 or HPLC-3FL-003
D-Lactose	? 5.0 w/w %	Glycom method HPAEC-HMO-021
3-Fucosyllactulose	? 1.5 w/w %	Glycom method HPAEC-HMO-021
Sum of other carbohydrates	? 5.0 w/w %	Glycom method HPAEC-HMO-021 + HPLC-3FL-003
pH in 5% solution (20°C)	3.2–7.0	Ph. Eur. 9.2 2.2.3 (07/2016:20203)
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 %	Megazyme K-ACETRM
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
Arsenic	? 0.2 mg/kg	EN 13805; EPA-6020A
Aflatoxin M1	0.025 µg/kg	LC-MS/MS Internal method Neotron 2015 Rev 1
Aerobic mesophilic total plate count	? 1,000 CFU/g	ISO 4833-1 or ISO-4833-2
Enterobacteriaceae	Absent in 10g	ISO 21528-2
Salmonella	Absent in 25 g	ISO 6579 or AFNOR BRD 07/11-12/05
Bacillus cereus	? 50 CFU/g	ISO 7932
Listeria monocytogenes	absent in 25g	ISO 11290-1
Cronobacter spp.	absent in 10g	ISO 22964
Yeasts	? 100 CFU/g	ISO 21527-2
Moulds	? 100 CFU/g	ISO 21527-2

[a] Specified saccharides include 3-fucosyllactose, D-lactose, L-fucose and 3-fucosyl-lactulose.
[b] Relevant only for crystallised 3-FL

33. The information provided is sufficient for the specification of 3-FL, and appropriately characterises the novel food seeking authorisation.

History of Use

34. There is no evidence for a history of use for 3-FL as a food ingredient.

35. Human breast milk contains a family of structurally related oligosaccharides, known as human milk oligosaccharides (HMOs), as the third largest solid components (Kunz and Rudloff, 1993; Bode, 2012; Newburg, 2013). The concentrations of HMOs in human colostrum are 20 to 25 g/L, whereas in mature human milk, the concentrations are 5 to 20 g/L (Bode, 2012). A wide variability has been reported in these values, depending on the individual, lactation period and genotype of the mother. Although there are over 140 known HMOs (Urashima et al., 2011; Chen, 2015; Remoroza et al., 2020), the five most abundant HMOs, on average, account for nearly half of the oligosaccharide fraction by mass. These are 2'-FL, LNFP-I, lacto-N-difucohexaose I (LNDFH-I), LNT, and the novel food, 3-fucosyllactose (3-FL) (Thurl et al., 2017; Molnar-Gabor et al., 2019).

36. A literature review of the quantitative data for 3-FL in human breast milk from forty-one publications reported that mean concentrations vary from 0.02 g 3-FL/L (van Niekerk et al., 2014) to 3.43 g 3-FL/L (Lefebvre et al., 2020). Using the reported standard deviations, the values reported by Gabrielli et al. (2011) represented the highest extrapolated 95% confidence limit (CL) at 4.72 g 3-FL/L. The estimated highest mean and highest 95% CL intake levels of 3-FL from human breast milk are reported in Table 6.

Table 6: Highest Intakes of 3-FL from 800ml and 1200ml of Breast milk for 6.7kg Infant [a]

Human Milk Oligosaccharide: 3-FL	Estimated highest intake for 800ml [b] milk (mg/kg bw/day)	Estimated highest intake for 1200ml [b] milk (mg/kg bw/day)
Highest Mean (based on 3.43 g/L)	410	614
Highest 95% CL (based on 4.72 g/L)	564	845

37. The history of use data did not indicate any further areas for evaluation.

Proposed Use and Anticipated Intake

38. Infants, children, and adults, including pregnant and lactating women, were identified as the target population of the novel food. The proposed food categories and maximum use levels are listed in Table 7.

Table 7: Food Categories and Use Levels for 3-FL from the novel food

Dairy Products and Analogues

Food Category Name	Proposed Maximum Use Level
Unflavoured pasteurised and unflavoured sterilised (including UHT) milk	2.0 g/L
Unflavoured fermented milk-based products	2.0 g/L (beverages) 4.0 g/kg (products other than beverages)
Flavoured fermented milk-based products including heat-treated products	2.0 g/L (beverages) 12.0 g/kg (products other than beverages)

Bakery Wares

Food Category Name	Proposed Maximum Use Level
Fine bakery wares. Cereal bars only	25.0g/kg

Foods for Special Groups (FSG)

Foods for infants and young children

Food Category Name	Proposed Maximum Use Level
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
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
Milk-based drinks and similar products intended for young children	2.0 g/L (beverages) in the final product ready for use, marketed as such or reconstituted as instructed by the manufacturer 12 g/kg (products other than beverages)

Foods for special medical purposes as defined in Regulation (EU) No 609/2013

Food Category Name	Proposed Maximum Use Level
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

Total diet replacement for weight control as defined in Regulation (EU) No 609/2013

Food Category Name	Proposed Maximum Use Level
Total diet replacement for weight control as defined in Regulation (EU) No 609/2013	2.0 g/L (beverages) 25.0 g/kg (products other than beverages)

Beverages

Food Category Name	Proposed Maximum Use Level
Flavoured drinks	1.25 g/L

Food Supplements

Food Category Name	Proposed Maximum Use Level
Food supplements (infants and young children)	2 g/day
Food supplements (other children, adolescents and adults)	4 g/day

39. The anticipated intake for 3-FL in children up to the age of 16 weeks is estimated to be 520 mg/kg body weight/day, equivalent to 3.48 g/day for a 6.7 kg infant. This value was calculated from the use of 3-FL in infant formula (2.0 g/L) at a high consumption level of 260 ml/kg body weight/day, as established by the EFSA Scientific Committee (EFSA SC, 2017). This value does not exceed the estimated intake for 3-FL at the highest 95% CL for breast milk (see Table 6).

40. An intake assessment using the summary statistics of consumption from the dietary surveys in the EFSA Comprehensive database was conducted by matching the proposed conditions of use with the FoodEx2 categories. The estimated mean and high-level intakes of 3-FL from the proposed conditions of use for each sub-population are presented in Table 8.

Table 8: Estimated daily intake of 3-FL from proposed food uses

Population Group	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 [a] (mg/kg bw/day)
Infants (? 11 months)	377 to 1,148	1,352 to 3,028	44 to 196	155 to 499
Young children (12 to 35 months)	473 to 1,189	983 to 2,373	38 to 119	92 to 231
Other children (3 to 9 years)	395 to 1,262	883 to 2,002	13 to 66	34 to 116
Adolescents (10 to 17 years)	185 to 1,326	716 to 2,381	3 to 25	16 to 49
Adults (18 to 64 years)	157 to 967	697 to 2,176	2 to 13	11 to 29
Pregnant and lactating women	326 to 907	906 to 1,789	5 to 13	12 to 28
Elderly (65 to 74 years)	151 to 777	605 to 2,195	2 to 10	10 to 27
Very elderly (75+ years)	117 to 835	737 to 1,487	2 to 11	13 to 20

[a] Results are not presented that were not statistically reliable (n < 60).

41. On a body weight basis, the highest intakes of 3-FL were observed in infants and young children. Higher intakes in these age groups are expected given the relatively high intake of foods and beverages on a body weight basis compared to the other population groups, where the intake levels are significantly lower. The wide range of high intake levels for 3-FL in infants and young

children represent the variability in the national survey data, and the assumption that all the proposed foods will be consumed at the maximum proposed intake levels. This represents an extremely unlikely scenario.

42. A comparison of the estimated mean and high level intake of 3-FL (based on all infants) against the highest mean and upper confidence limit exposures from breast milk for 3-FL on a body weight basis (EFSA NDA Panel, 2013), are shown in Table 9. This data indicates that the estimated intake of 3-FL, in the most sensitive population group (infants), is not expected to exceed the levels found in human breast milk, for which there is a history of safe use.

Table 9: Highest Intakes of 3-FL from Breast Milk versus Highest Level of Exposure from Proposed Uses in Infants

Mean Intake of Human Milk (800 ml) for 6.7 kg infant

Dietary Source	Estimated Daily Intake for 3-FL (mg/kg bw/day)
Highest Mean (based on 3.43 g/L [a])	410
Highest 95% CL (based on 4.72 g/L [a])	564
Highest Mean Consumption from Proposed Uses [b]	196

Mean Intake of Human Milk (1200 ml) for 6.7 kg infant

Dietary Source	Estimated Daily Intake for 3-FL (mg/kg bw/day)
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

[a] Calculated using quantitative analytical data for oligosaccharides in breast milk
[b] Estimated intake for all infants.

43. The use level for 3-FL in food supplements is 2 g/day for infants and young children, and 4 g/day for all other population sub-groups. Food supplements are not intended to be used if other foods with the novel food are consumed on the same day. For infants and young children, food supplements are not intended to be used if breast milk or other foods with added 3-FL are consumed on the same day.

44. The estimated intake for 3-FL in food supplements on a body weight basis for all population groups at the proposed use levels is presented in Table 10.

Table 10: Estimated daily intake of 3-FL from food supplements

Proposed use level (g/day)	Population Group	Mean Body Weight (kg) [a]	Estimated intake (mg/kg bw/day) [b]
2.0	Infants (? 11 months)	6.7	299
2.0	Young children (12 to 35 months)	11.9	168
4.0	Other children (3 to 9 years)	23.1	173
	Adolescents (10 – 17 years)	43.4	92
	Adults (18 – 64 years)	73.9	54
	Elderly (65 – 74 years)	76.0	53
	Very Elderly (? 75 years)	71.2	56

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

45. The estimated mean and high-level intake of 3-FL in food supplements (based on infants) against the highest mean and upper confidence limit exposures from breast milk for 3-FL on a body weight basis is shown in Table 11. The results confirm that the estimated intake of 3-FL in food supplements is not be expected to exceed the levels found in human breast milk, for which there is a history of safe use.

Table 11: Highest Intakes of 3-FL from Breast Milk versus 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	564 mg/kg bw/day	614 mg/kg bw/day	845 mg/kg bw/day	299 mg/kg bw/day

Highest Mean (based on 3.43 g/L)
Highest 95% CL (based on 4.72 g/L)

Absorption, Distribution, Metabolism and Excretion (ADME)

46. The 3-FL oligosaccharide in the novel food has the same structure as the naturally occurring counterpart in human breast milk.

47. Most human milk oligosaccharides are reported to undergo limited oral absorption intact. Human milk oligosaccharides do not undergo significant digestion in the upper gastrointestinal tract but can undergo fermentation in the colon. Human milk oligosaccharides are predominantly excreted unchanged in the faeces, with a small proportion excreted unchanged in the urine.

48. The absorption of 3-FL from consumption of the novel food is not expected to differ from the intake of human milk oligosaccharides following infant consumption of breast milk. Therefore, this was not expected to pose a safety concern for infants or other age groups.

49. Committee members noted that oligosaccharides are usually considered prebiotic and can cause bloating in high doses. Consequently, it was highlighted that risk managers may wish to consider whether there was a need for foods containing 3-FL to be labelled on this basis.

50. The ADME of human milk oligosaccharides are well understood and the information does not indicate any further areas of concern.

Nutritional information

51. The novel food is mainly composed of the oligosaccharide, 3-FL, which is structurally identical to the naturally occurring counterpart in human breast milk. Consumption of the novel food at the proposed use levels is not expected to be nutritionally disadvantageous for consumers.

Toxicological information

52. Toxicological studies were performed with 3-FL to support the safety assessment of the novel food. The respective study reports are unpublished and claimed as proprietary data. They were considered essential in the assessment the safety of the novel food and were reviewed by the

ACNFP.

53. In vitro genotoxicity testing of 3-FL was conducted under Good Laboratory Practice (GLP) conditions and according to the following OECD guidelines: in vitro bacterial reverse mutation test (OECD TG 471) and in vitro mammalian cell micronucleus test (OECD TG 487). This approach is recommended by the UK Committee on Mutagenicity, and is also the basis of guidance on the preparation and submission of an application for authorisation of a novel food in the context of Regulation (EU) 2015/2283, as retained in UK law.

54. The in vitro bacterial reverse mutation test (Gilby, 2020a) demonstrated that 3-FL is non-mutagenic, in the absence or presence of metabolic activation.

55. The in vitro mammalian cell micronucleus test (Gilby, 2020b) demonstrated that 3-FL is non-clastogenic and non-aneugenic in the absence or presence of metabolic activation.

56. The results from these in vitro studies support the conclusion that the novel food is not genotoxic.

57. Repeated Dose 90-Day Oral Toxicity Study in Rodents (Stannard, 2020) was conducted under GLP conditions according to OECD TG 408 guidelines as recommended by the Guidance on the preparation and submission of an application for authorisation of a novel food in the context of Regulation (EU) 2015/2283, as retained in UK law. The aim of the study was to identify any adverse effects following the consumption of 3-FL.

58. In this 90-day feeding study, each group consisted of 10 female and 10 male rats which were fed 0 (control – vehicle only [water]), 1,000, 3,000 or 4,000 mg/kg bw/day of 3-FL by oral gavage. A reference control group consisting of the same number of animals was fed oligofructose (4,000 mg/kg bw/day). Additionally, satellite groups consisting of 5 female rats and 5 male rats for the control and high dose treatment, and the reference control treatment were used as recovery groups.

59. No deaths, test item-related clinical abnormalities, ocular changes, or differences in food consumption and bodyweight between test groups were reported. There were no statistically significant dose dependent changes in haematology, blood chemistry, urinalysis, serum hormone levels, or organ weights. In addition, no dose related abnormalities were noted during the necropsy or histopathological evaluation. Therefore, the no observable adverse effect level (NOAEL) for 3-FL was considered to be the high-dose of 4,000 mg/kg bw/day.

Allergenicity

60. The protein content of the novel food is reported as < 0.01% w/w. The potential allergenicity of the introduced proteins expressed in *E. coli* K-12 (Allergen Online tool, version 21 – University of Nebraska) was assessed. None of the proteins was predicted to be an allergen.

61. The novel food is unlikely to trigger allergic reactions in the target population under the proposed conditions of use.

Discussion

62. The novel food is a human identical milk trisaccharide containing ? 90.0% dry weight of 3-FL, which is manufactured by microbial fermentation using a genetically modified strain of *Escherichia coli* K-12. The total saccharide content of the novel food is up to 92% dry weight including of other saccharides present in smaller quantities.

63. 3-FL is intended to be used in dairy products and analogues, bakery wares, beverages, foods for infants and young children, foods for special medical purposes, total diet replacement for weight control, and food supplements. Infants, children, and adults, including pregnant and lactating women, are identified as the target population of the novel food.

64. Analysis confirms that the novel food is structurally identical to the 3-FL found naturally in human milk. Exposure to 3-FL relates solely to breastfeeding infants as there is no recognised history of use for this milk oligosaccharide as an ingredient in foods or food supplements.

65. In the Repeated Dose 90-Day Oral Toxicity Study in Rodents, the NOAEL for 3-FL was 4,000 mg/kg bw/day, the highest dose tested. When this NOAEL is compared with the highest estimated exposure in each population category, the margins of exposure range from 8 to 148. Given that the 3-FL in the novel food is equivalent to 3-FL found in human breast milk, these margins of exposure are acceptable with respect to the highest estimated daily intakes in the intended population.

66. Moreover, the anticipated daily intake of the novel food in all population groups, including children up to the age of 16 weeks using infant formula alone, is not expected to exceed the highest intake level of 3-FL in breastfed infants on a body weight basis.

67. The use level of 3-FL in food supplements (2 g/day for infants and young children, and 4 g/day for all other population sub-groups) is not expected to exceed the highest intake level of 3-FL in breastfed infants on a body weight basis. Food supplements are not intended to be used if other foods containing the novel food, including breast milk or other foods for infants and young children, are consumed on the same day. It is noted that the concurrent use of 3-FL in foods and food supplements by adolescents and adults would not be expected to exceed the intake levels of 3-FL in breastfed infants on a bodyweight basis.

Conclusions

68. The FSA and FSS have undertaken the assessment of 3-FL and concluded that the composition of the novel food is safe under the proposed conditions of use and does not pose a safety risk to human health.

69. These conclusions were based on the information in the novel food dossier submitted by the applicant plus the supplementary information and could not have been reached without the following data claimed as proprietary by the applicant:

- annexes to the dossier which relate to the identity of the novel food, the production process, composition, stability, history of use, and the anticipated intake of the novel food.
- bacterial reverse mutation test (Gilby, 2021a [unpublished]), in vitro micronucleus test (Gilby, 2021b [unpublished]), 14-day dose range finding study (Stannard, 2021a [unpublished]) and 90-day repeat dose feeding study with the novel food (Stannard, 2021b [unpublished]) including the summary table of statistically significant observations in the toxicity studies (Appendix B.3).

References

- Bode L, 2012. Human milk oligosaccharides: every baby needs a sugar mama. Glycobiology, 22(9), 1147-1162. Human milk oligosaccharides: Every baby needs a sugar mama
- Chen X, 2015. Human milk oligosaccharides (HMOS): structure, function, and enzyme-catalyzed synthesis. Advances in Carbohydrate Chemistry and Biochemistry, 72, 113-190. Human Milk Oligosaccharides (HMOS): Structure, Function, and Enzyme-Catalyzed Synthesis

- Christensen AS, Skov SH, Lendal SE and Hornshøj BH, 2020. Quantifying the human milk oligosaccharides 2'-fucosyllactose and 3-fucosyllactose in different food applications by high-performance liquid chromatography with refractive index detection. Journal of Food Science, 85(2), 332-339. Quantifying the human milk oligosaccharides 2'-fucosyllactose and 3-fucosyllactose in different food applications by high-performance liquid chromatography with refractive index detection
- EC, 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. COMMISSION IMPLEMENTING REGULATION (EU) 2017/2469
- EFSA, 2011 GMO Panel (EFSA Panel on Genetically Modified Organisms). Scientific Opinion on Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use (Question no EFSA-Q-2009-00521, adopted: 25 May 2009). EFSA Journal, 9(6):2193 [54 pp.]. Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use
- EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2013. Scientific Opinion on nutrient requirements and dietary intakes of infants and young children in the European Union. EFSA Journal, 11(10):3408 [103 pp.]. Scientific Opinion on nutrient requirements and dietary intakes of infants and young children in the European Union
- EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2021. Guidance on the preparation and submission of an application for authorisation of a novel food in the context of Regulation (EU) 2015/2283 (Revision 1). Published 26 March 2021. Scientific and technical guidance for the preparation and presentation of an application for authorisation of a health claim
- EFSA Scientific Committee, 2012. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 10(3):2579. [32 pp.] Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data
- EFSA Scientific Committee, 2017. Guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age (Question no EFSA-Q-2016-00489, adopted: 26 April 2017). EFSA Journal, 15(5):4849 [58 pp.]. Guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age
- EU (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 1852/2001Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods
- Gabrielli O, Zampini L, Galeazzi T, Padella L, Santoro L, Peila C, Giuliani F, Bertino E, Fabris C and Coppa GV, 2011. Preterm milk oligosaccharides during the first month of lactation. Pediatrics, 128(6), e1520-e1531. Preterm Milk Oligosaccharides During the First Month of Lactation
- Gilby B, 2021a [unpublished]. Dated: 17 February 2021. Covance Laboratories Limited, Huntingdon, Cambridgeshire, UK. Study Title: Report. 3-Fucosyllactose (3-FL): Bacterial reverse mutation test. Confidential. (Covance Study Number: 8429603).
- Gilby B, 2021b [unpublished]. Dated: 17 February 2021. Covance Laboratories Limited, Huntingdon, Cambridgeshire, UK. Study Title: Report. 3-Fucosyllactose (3-FL): In Vitro micronucleus test in human lymphocytes. Confidential. (Covance Study Number: 8436271).
- Kunz C and Rudloff S, 1993. Biological functions of oligosaccharides in human milk. Acta Paediatrica, 82(12), 903-912. Biological functions of oligosaccharides in human milk
- Lefebvre G, Shevlyakova M, Charpagne A, Marquis J, Vogel M, Kirsten T, Kiess W, Austin S, Sprenger N and Binia A, 2020. Time of lactation and maternal fucosyltransferase genetic polymorphisms determine the variability in human milk oligosaccharides. Frontiers in

Nutrition, 7, Article 574459 [12pp, plus supplementary data]. Time of Lactation and Maternal Fucosyltransferase Genetic Polymorphisms Determine the Variability in Human Milk Oligosaccharides
- Molnar-Gabor D, Hederos MJ, Bartsch S and Vogel A, 2019. Emerging field – Synthesis of complex carbohydrates. Case study on HMOs (Chapter 2.5). In: Industrial enzyme applications. Eds Vogel A and, May O. Wiley-VCH Verlag GmbH, Weinheim, Germany, 179-201. Emerging Field – Synthesis of Complex Carbohydrates. Case Study on HMOs
- Newburg DS, 2013. Glycobiology of human milk. Biochemistry (Moscow), 78, 771-785 (Russian edition "Biokhimiya, 778, 990-1007"). Glycobiology of human milk
- OECD (Organisation for Economic Co-operation and Development), 1997. Bacterial reverse mutation test. In OECD guidelines for the testing of chemicals. OECD guideline No 471 (updated & adopted: 21 July 1997). Paris, France: Organisation for Economic Co-operation and Development (OECD). OECD Guidelines for the Testing of Chemicals, Section 4
- OECD (Organisation for Economic Co-operation and Development), 1998. OECD principles of good laboratory practice. Series on principles of good laboratory practice and compliance monitoring, No. 1 (ENV/MC/CHEM(98) 17). Paris, France: Organisation for Economic Co-operation and Development (OECD), Environment
- Directorate, Chemicals Group and Management Committee. OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring
- OECD (Organisation for Economic Co-operation and Development), 2016. In vitro mammalian cell micronucleus test. In OECD guidelines for the testing of chemicals. OECD guideline No 487 (updated & adopted: 29 July 2016). Paris, France: Organisation for Economic Cooperation and Development (OECD).
- OECD Guidelines for the Testing of Chemicals, Section 4
- OECD (Organisation for Economic Co-operation and Development), 2018. Repeated dose 90-day oral toxicity study in rodents. In OECD guidelines for the testing of chemicals. OECD guideline No 408 (updated and adopted 27 June 2018). Paris, France: Organisation for Economic Cooperation and Development (OECD). OECD Guidelines for the Testing of Chemicals, Section 4
- Remoroza CA, Liang Y, Mak TD, Mirokhin Y, Sheetlin SL, Yang X, San Andres JV, Power ML and Stein SE, 2020. Increasing the coverage of a mass spectral library of milk oligosaccharides using a hybrid-search-based bootstrapping method and milks from a wide variety of mammals. Analytical Chemistry, 92(15), 10316-10326.Increasing the Coverage of a Mass Spectral Library of Milk Oligosaccharides Using a Hybrid-Search-Based Bootstrapping Method and Milks from a Wide Variety of Mammals
- Stannard DR, 2021a [unpublished]. Dated 17 February 2021. Covance Laboratories Limited, Suffolk, UK. Study Title: Final report. 3-Fucosyllactose (3-FL): 14-Day dose range finding study in the neonatal Crl:CD(SD) rat by oral (gavage) administration. Confidential. (Covance Study Number: 8438088).
- Stannard DR, 2021b [unpublished]. Dated: TBD. Covance Laboratories Limited, Suffolk, UK. Study plan: 3-Fucosyllactose (3-FL): 90-Day toxicity study in the neonatal Crl:CD(SD) rat by oral (gavage) administration followed by a 4-week recovery period. Draft report. Confidential. (Covance Study Number: 8437221).
- Thurl S, Munzert M, Boehm G, Matthews C and Stahl B, 2017. Systematic review of the concentrations of oligosaccharides in human milk. Nutrition Reviews, 75(11), 920-933. Systematic review of the concentrations of oligosaccharides in human milk
- Townsend S, Caubilla Barron J, Loc-Carrillo C, Forsythe, S (2007). The presence of endotoxin in powdered infant formula milk and the influence of endotoxin and Enterobacter sakazakii on bacterial translocation in the infant rat. Food Microbiology, 24(1), 67-74. The presence of endotoxin in powdered infant formula milk and the influence of endotoxin and *Enterobacter sakazakii* on bacterial translocation in the infant rat
- Urashima T, Fukuda K, Kitaoka M, Ohnishi M, Terabayashi T and Kobata A, 2011. Milk oligosaccharides. Nova Biomedical Books, New York, Nutrition and diet research progress.

- van Niekerk E, Autran CA, Nel DG, Kirsten GF, Blaauw R and Bode L, 2014. Human milk oligosaccharides differ between HIV-infected and HIV-uninfected mothers and are related to necrotizing enterocolitis incidence in their preterm very-low-birth-weight infants. The Journal of Nutrition, 144(8), 1227-1233
- Human Milk Oligosaccharides Differ between HIV-Infected and HIV-Uninfected Mothers and Are Related to Necrotizing Enterocolitis Incidence in Their Preterm Very-Low-Birth-Weight Infants