

Safety Assessment of 3-Fucosyllactose (3-FL) as a Novel Food for Use in Food and Food Supplements (RP2001)

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FSA Research and Evidence

The Food Standards Agency (FSA) and Food Standards Scotland (FSS) received an application from Inbiose N.V., Belgium (“the applicant”) for the authorisation of 3-fucosyllactose (3-FL) as a novel food in April 2023.

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

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

The FSA and FSS in their evaluation of the application reviewed the safety dossier and supplementary information provided by the applicant. The FSA and FSS did not consider any potential health benefits or claims arising from consuming the food, as the focus of the novel food assessment is to ensure the change in the conditions of use to extend the food is safe and not putting consumers at a nutritional disadvantage.

The FSA and FSS concluded that the applicant had provided sufficient information to assure that 3-FL was safe under the intended conditions of use. The anticipated intake levels and the intended use in food and food supplements was not considered to be nutritionally disadvantageous.

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

This is a joint FSA and FSS publication.



1. Introduction

In April 2023, Inbiose N.V., Belgium ("the applicant") submitted a full novel food application to the FSA and FSS for the authorisation of 3-fucosyllactose (3-FL). The novel food is a water-soluble white to off-white powder composed of $\geq 90.0\%$ w/w dry matter (DM) of 3-FL, which is manufactured by microbial fermentation using a genetically modified strain of *Escherichia coli* K-12. The 3-FL is intended to be used as a source of human identical milk oligosaccharide.

The FSA and FSS have undertaken a safety assessment for 3-FL under the novel foods legislation, assimilated Regulation (EU) 2015/2283.

The evaluation by the FSA and FSS assessed the food safety risks of the novel food and its production, in line with Article 11 of assimilated Regulation (EU) 2015/2283 and Article 7 of assimilated Commission Implementing Regulation (EU) 2017/2469. The basis and structure of the assessment was conducted in accordance with the relevant technical guidance put in place by the European Food Safety Authority (EFSA) for full novel food applications (EFSA NDA Panel, 2016), which the FSA and FSS considers is relevant and should be applied to this novel food application owing to similarities in the regulatory regimes.

This safety assessment outlines the conclusions of the FSA and FSS on the safety of 3-FL as a novel food.

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

2. Assessment

2.1. Identity of the novel food

The novel food is a purified white to off-white powder containing $\geq 90\%$ 3-FL by dry weight (DM). Other saccharides are present in smaller quantities: D-lactose ($\leq 5\%$ w/w DM); L-fucose ($\leq 3\%$ w/w DM); galactose and glucose ($\leq 3\%$ w/w DM); other carbohydrates ($\leq 3\%$ w/w DM).

3-FL is a trisaccharide composed of L-fucose, D-galactose and D-glucose (Figure 1), with the L-fucose linked to D-glucose by an α -(1-3) bond. This is identical to the structure of 3-FL in human breast milk.

3-FL is a constitutional isomer of 2'-fucosyllactose (2'-FL). Both isomers contain the same monosaccharides but there is an α -(1-2') bond between L-fucose and the D-galactose moiety of D-lactose in 2'-FL.

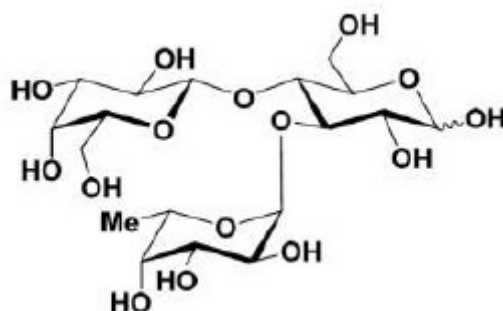


Figure 1. The structural formula of 3-fucosyllactose (3-FL)

The novel food is characterised by the following information: molecular formula $C_{18}H_{32}O_{15}$; molecular mass 488.48 Daltons; CAS number 41312-47-4; and IUPAC name 6-deoxy- α -L-galacto-hexopyranosyl-(1 $\rightarrow$ 3)-[β -D-galacto-hexopyranosyl-(1 $\rightarrow$ 4)]-D-glucopyranose.

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: 1H -NMR and 2D 1H - ^{13}C heteronuclear single quantum correlation (HSQC) and heteronuclear multiple bond correlation (HMBC) analysis. Furthermore, long range 1H and ^{13}C NMR correlations in the HBMC spectra unequivocally confirmed that the fucose was linked to glucose by an α -(1-3) bond.

Hydrophilic liquid interaction chromatography – refractive index (HILIC-RI) was used to characterise 3-FL in five batches of novel food.

2.2. Production process

The production microorganism used to manufacture the novel food, *Escherichia coli* K-12 MG1655 INB001084 (deposited BCCM/LMG Collection, Belgium) of *Escherichia coli* K-12 MG1655 that functions as a processing aid as defined in Article 3(2)(b) of assimilated Regulation (EC) No.1333/2008 on food additives. A novel food produced by a GMO does not fall under the remit of the GMO legislation, assimilated Regulation (EC) No 1829/2003 or assimilated Regulation (EC) No 1830/2003, when the production

microorganism is removed during the manufacturing process and therefore no recombinant DNA remains. This has been confirmed in the compositional analysis as detailed below ([Table 2](#)).

The novel food is classified as category 1 under the EFSA GMO guidance (EFSA GMO Panel, 2011): 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, and which the FSA have retained for the purposes of technical review.

Although *Escherichia coli* is not considered to be suitable for qualified presumption of safety (QPS) status (EFSA BIOHAZ Panel, 2023), *Escherichia coli* K-12 is widely used for biotechnological applications. Genomic analysis confirms that the genes required for pathogenicity are missing key components, or they have been mutationally inactivated (Blattner et al., 1997). Furthermore, *Escherichia coli* K-12 is considered to be a safe and non-pathogenic microorganism because this microbe does not cause disease in healthy adult humans or colonise the human gut (Gorbach, 1978; Muhldorfer and Hacker, 1994; OECD, 1986; U.S. EPA, 1997). On the basis of this information, the new production strain organism does not introduce any new risks that need to be evaluated and managed.

The absence of bacteria from the *Enterobacteriaceae* family (ISO 21528-1) and residual bacterial DNA (LOD = 10 ng DNA/g of novel food) confirms the genetically modified *Escherichia coli* K-12 is not present in the novel food.

The first stage of the production process involves the conversion of D-lactose and D-sucrose to 3-FL by the adapted cellular metabolism of the production microorganism. Sucrose 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 *Escherichia coli* K-12 into the fermentation broth. The production microorganism, *Escherichia coli* K-12, is removed from the culture medium by filtration at the end of the fermentation process.

The second stage of the production process involves a series of purification and isolation steps (filtration, ion exchange, concentration and spray drying) to obtain the final purified novel food in powder form.

Information on the acceptance criteria for the raw materials and processing aids was provided. The purity criteria for the reagents used in the manufacture of 3-FL are listed in [Table 1](#).

Table 1. Purity criteria for reagents used to synthesize the novel food

Raw material	Purity
D-sucrose	≥ 99.9% saccharose
D-lactose	≥ 99% lactose

The novel food is produced in line with Hazard Analysis and Critical Control Point (HACCP) principles.

Bacterial contamination of the novel food is controlled by sterile filtration. To prevent bacterial growth, most purification steps are conducted at low temperatures and the water content in the finished product is specified at $\leq 5\%$ w/w. Endotoxin levels in the novel food are controlled by filtration and compliance with the specified level at ≤ 300 endotoxin units (EU)/g.

The production process has characterised the potential hazards, and the corresponding control measures are appropriate.

2.3. Compositional information

Results from five independent batches ([Table 2](#)) demonstrate that the novel food is appropriately characterised.

The novel food primarily contains 3-FL with small quantities of other carbohydrates present. Lactose is the most prevalent molecule in human milk (approximately 7 g/100 mL). Galactose and glucose, which are monomers of lactose, are normal constituents of human milk. L-fucose is also found in human milk (Smilowitz et al., 2013) at concentrations ranging from 20 to 30 mg/L (Choi et al., 2015). In representative batches of the novel food, the average amount of lactose and other carbohydrates was 1.7% (0.2 – 2.9%) and 1.8% (1.2 – 2.7%), respectively.

The fermentation medium contains minerals and trace elements, with trace metals functioning as co-factors for different enzymes. Analysis confirmed the presence minerals at low levels in three independent batches of the novel food. Sodium was present in batches 1, 3 and 5 of the novel food (18, 6 and 21 mg/kg, respectively). In addition, potassium was present in batch 1 (25 mg/kg), copper was present in batch 3 (0.2 mg/kg), and calcium and magnesium were present in batch 5 (4 mg/kg and 1.6 mg/kg, respectively).

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.

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

2.4. Stability

Stability tests were conducted with batches 1 to 5 of the novel food under normal storage conditions (25°C and 60% RH) in commercial packaging for up to 24 months. Each batch was analysed for 3-FL, lactose, galactose + glucose, fucose, other carbohydrates and moisture. Microbiological

Table 2. Compositional Analysis of five independent batches of novel food

Parameter	Specification	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5
3-fucosyllactose (% w/w DM)	≥ 90	91.5	90.8	93.4	94.1	93.2
D-lactose (% w/w DM)	≤ 5	2.2	2.9	0.2	1.1	2.3
L-fucose (% w/w DM)	≤ 3	1.1	0.8	1.9	0.3	0.3
Galactose/Glucose (% w/w DM)	≤ 3	1.2	1.0	2.0	0.5	0.4
Other carbohydrates (% w/w DM) *	≤ 3	2.0	2.7	1.2	1.6	1.5
Total carbohydrates (% w/w DM)		98.0	98.2	98.7	97.7	97.7
Water content (%)	≤ 5.0	3.42	3.52	3.7	3.2	3.0
pH (5% solution)	3.0 – 7.5	6.0	6.1	5.5	6.3	5.5
Protein content (µg/g)	≤ 100	≤ 25	≤ 25	≤ 25	≤ 25	≤ 25
Total Ash (%)	≤ 0.5	< 0.12	< 0.12	< 0.12	< 0.12	< 0.12
Arsenic (mg/kg)	≤ 0.2	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Cadmium (mg/kg)	≤ 0.05	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Lead (mg/kg)	≤ 0.05	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
Mercury (mg/kg)	≤ 0.1	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Aflatoxin M1 (µg/kg)	≤ 0.025	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Aflatoxin B1 (µg/kg) **	≤ 0.1	< 1	< 1	< 0.1	< 1	< 1
Endotoxins (EU/1,000 mg)	300	15.7	29.8	14.4	157	7.9
GMO detection (ng DNA/g) ***	< 10 ng DNA/g	Not detected	Not detected	Not detected	Not detected	Not detected
Standard Plate Count (CFU/g)	≤ 1,000	190	< 10	< 10	< 10	< 10
Yeast (CFU/g)	≤ 100	< 10	< 10	< 10	< 10	< 10
Mould (CFU/g)	≤ 100	< 10	< 10	< 10	< 10	< 10
Enterobacteriaceae (in 10g)	Absent in 10 g	Absent in 10 g	Absent in 10 g	Absent in 10 g	Absent in 10 g	Absent in 10 g
<i>Salmonella</i> spp. (in 100g)	Absent in 100 g	Absent in 100 g	Absent in 100 g	Absent in 100 g	Absent in 100 g	Absent in 100 g
<i>Cronobacter sakazakii</i> (in 100g)	Absent in 100 g	Absent in 100 g	Absent in 100 g	Absent in 100 g	Absent in 100 g	Absent in 100 g
<i>Listeria monocytogenes</i> (in 25g)	Absent in 25 g	Absent in 25 g	Absent in 25 g	Absent in 25 g	Absent in 25 g	Absent in 25 g
<i>Bacillus cereus</i> (CFU/g)	≤ 10	< 10	< 10	< 10	< 10	< 10

CFU: colony forming units; DIN = German Institute for Standardization; DM: dry matter; EN = English; EU: endotoxin units; GMM: genetically modified microorganisms; GMO: genetically modified organism; HPLC-HILIC/RI = hydrophilic liquid interaction chromatography – refractive index; ISO = International Organisation for Standardization; L mono = *Listeria monocytogenes*; NMKL = Nordic-Baltic Committee on Food Analysis; LoD: limit of detection; LoQ: limit of quantification; Ph. Eur. = European Pharmacopoeia; TS = technical standard

* Includes 3-FL isomers, difucosyllactose isomer and carbohydrate oligomers

** For batch 3, a different sample matrix category characterised was used (LoQ < 0.1 µg/kg)

*** The GMO detection means absence of DNA from the GMM strain (LoD < 10 ng DNA/g of novel food)

3-fucosyllactose, D-lactose, L-glucose, galactose/glucose, and other carbohydrates – HPLC-HILIC/RI; water content – Karl-Fischer titration (ISO 8534:2017); pH – potentiometric measurement; protein content – Nanoquant (modified Bradford); total ash – NMKL 173:2005, modified; heavy metals – DIN EN 15763:2010; aflatoxin M1 – EN ISO 14501; aflatoxin B1 – DIN EN 14123 mod; endotoxins – Ph. Eur. 2.6.14 + Interference study; GMO – internal method; standard plate count – ISO 4833-1; yeasts and moulds – NMKL 98; Enterobacteriaceae – ISO 21528-1; *Salmonella* spp. – NMKL 71; *Cronobacter sakazakii* – ISO/TS 22964; *Listeria monocytogenes* – ISO 11290-1/Rapid L mono; *Bacillus cereus* – ISO 7932/NMKL 67

analyses were conducted at time 0, in the middle and at the end of the stability test period. The results demonstrated that the content of 3-FL remained unchanged throughout the study.

An accelerated stability study at 40°C and at 75% RH over a 6-month period for the same five batches was also conducted. No changes were observed in the levels of carbohydrates, moisture or microbiological parameters during storage.

The novel food is expected to be stable for up to 24 months when stored at room temperature.

The stability of 3-FL as an ingredient in whole milk, UHT milk, yoghurt, and cereal bars was assessed. In the whole milk, the 3-FL content remained stable (0.97–1.1 mg/mL) for up to 22 days. In UHT milk, the content of 3-FL was stable over 91 days (first measured value of 0.87 on Day 7 to 0.84 mg/mL on Day 91). A small drop in the 3-FL content was noted between time 0 (3-FL content calculated as 0.95 mg/mL) and the first time point in the study. Since the 3-FL was added to the milk before the UHT processing (142°C for 3 seconds) this could explain the initial observed decrease in 3-FL content.

In yoghurt, only 72% of the initial 3-FL content was present after 35 days, with the largest decrease of approximately 25% recorded on day 1 of the study. The 3-FL was added to the yogurt prior to the pasteurisation process (95°C for 6 min). The decrease in the content of 3-FL could be the result of either pasteurisation or fermentation with starter cultures at a low pH (4.6). During the remaining 34 days of the study, the 3-FL content remained stable (Støvlbæk Christensen et al., 2020).

In cereal bars, approximately 100% of the initial 3-FL content was present after 4 months storage at ambient temperature. Similar results (94 – 100% of the original content) were also recorded after 6 weeks at accelerated storage conditions (32°C).

The data provided supports the stability of the novel food in the food matrices at neutral pH under proper storage conditions. However, acidic pH, and especially thermal treatments, may decrease the 3-FL content in finished food products.

2.5. Specification

The specification for the novel food ([Table 3](#)) was assessed using internationally recognised methods or determined using internally developed and validated methods.

Table 3. Specification for the novel food

Parameter	Specification	Method
3-fucosyllactose	≥ 90 % w/w DM	HPLC-HILIC/RI (internal)
D-lactose	≤ 5 % w/w DM	HPLC-HILIC/RI (internal)
L-fucose	≤ 3 % w/w DM	HPLC-HILIC/RI (internal)
Galactose/glucose	≤ 3 % w/w DM	HPLC-HILIC/RI (internal)
Other carbohydrates *	≤ 3 % w/w DM	HPLC-HILIC/RI (internal)
Water content (%)	≤ 5.0 %	Karl-Fischer titration (ISO 8534:2017)
pH (5% solution)	3.0 – 7.5	Potentiometric measurement
Protein content	≤ 100 µg/g	Nanoquant (modified Bradford)
Total ash	≤ 0.5 %	NMKL 173:2005 modified
Arsenic	≤ 0.2 mg/kg	DIN EN 15763:2010
Cadmium	≤ 0.05 mg/kg	DIN EN 15763:2010
Lead	≤ 0.05 mg/kg	DIN EN 15763:2010
Mercury	≤ 0.1 mg/kg	DIN EN 15763:2010
Aflatoxin M1	≤ 0.025 µg/kg	EN ISO 14501
Aflatoxin B1	≤ 0.1 µg/kg	DIN EN 14123 modified
Endotoxins	300 EU/1,000 mg	Ph. Eur. 2.6.14 + Interference study
GMO detection **	< 10 ng DNA/g	Internal method
Standard plate count	≤ 1,000 CFU/g	ISO 4833-1
Yeast	≤ 100 CFU/g	NMKL 98
Mould	≤ 100 CFU/g	NMKL 98
Enterobacteriaceae	Absent in 10 g	ISO 21528-1
<i>Salmonella</i> spp.	Absent in 100 g	NMKL 71
<i>Cronobacter sakazakii</i>	Absent in 100 g	ISO/TS 22964
<i>Listeria monocytogenes</i>	Absent in 25 g	ISO 11290-1/Rapid L mono
<i>Bacillus cereus</i>	≤ 10 CFU/g	ISO 7932/NMKL 67

DIN = German Institute for Standardization; DM = dry matter; EN = English; EU = Endotoxin unit; GMO = genetically modified organism; GMM = genetically modified microorganism; HPLC-HILIC/RI = hydrophilic liquid interaction chromatography – refractive index; ISO = International Organisation for Standardization; L mono = *Listeria monocytogenes*; LoD = limit of detection; NMKL = Nordic-Baltic Committee on Food Analysis; Ph. Eur. = European Pharmacopoeia; TS = technical standard

* Includes 3-FL isomers, difucosyllactose isomer and carbohydrate oligomers.

** The GMO detection means absence of DNA from the GMM strain (LoD < 10 ng DNA/g of novel food).

The information provided is sufficient for the specification of the novel food and appropriately characterises the novel food seeking authorisation.

2.6. History of use

There is no history of use for the novel food in the UK.

Although the novel food, 3-FL produced by genetically modified *Escherichia coli* K-12 MG1655, has been authorised in the EU (assimilated Commission Implementing Regulation (EU) 2017/2470), this application to the FSA and FSS includes additional food categories and different use levels.

Other novel foods, where 3-FL is the major constituent, have also been authorised in the EU (assimilated Commission Implementing Regulation (EU) 2017/2470). These novel foods were produced by fermentation using genetically modified *Escherichia coli* BL21(DE3) (Chr. Hansen, Denmark) or genetically modified *Escherichia coli* K-12 DH1 (Glycom A/S, Denmark). However, the authorised food categories and use levels for these novel foods differ from the current application.

Human breast milk contains a family of structurally related oligosaccharides, known as human milk oligosaccharides (HMOs), as the third largest solid components (Bode, 2012; Kunz & Rudloff, 1993; 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; Thurl et al., 2010; Urashima et al., 2018).

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 (Chen, 2015; Remoroza et al., 2020; Urashima et al., 2011), the five most abundant HMOs, on average, account for nearly half of the oligosaccharide fraction by mass. These are 2'-FL (2'-fucosyllactose), LNFP-I (lacto-N-fucopentaose I), lacto-N-difucohexaose I (LNDFH-I), LNT (lacto-N-tetraose), and the novel food, 3-FL (Molnar-Gabor et al., 2019; Thurl et al., 2017).

3-FL belongs to the subfraction of fucosylated HMOs (Bode, 2012; Rijnierse et al., 2011; Thurl et al., 2010) and is one of the most predominant HMOs in human milk, with a mean of mean concentration of 0.97 g/L and a maximum mean concentration of 5.00 g/L (EFSA, 2024).

Using the reported levels of 3-FL in human breast milk from EFSA (2024) and considering the average and high daily intake of breast milk (800 mL and 1,200 mL, respectively) for infants from 0 to 6 months (EFSA NDA Panel, 2013), the estimated daily intake levels of 3-FL from human milk for a 6.7 kg body weight infant (EFSA SC, 2012) are shown in [Table 4](#).

Table 4. Estimated intakes of 3-FL from 800mL and 1200mL of Breast milk for 6.7kg Infant * using data from EFSA (2024)

	Mean intake levels (mg/kg BW/day)	95 th percentile intake levels (mg/kg BW/day)
Estimated highest intake for 800 mL milk **	109	597
Estimated highest intake for 1,200 mL milk **	163	896

* EFSA Scientific Committee (2012)

** EFSA NDA Panel (2013)

The history of use does not indicate any further areas for evaluation.

2.7. Proposed use and intake

Foods containing the novel food are intended for the general population. Food supplements are intended to be consumed by all population groups, except for infants.

The intended use and maximum use level for the novel food in each food category is shown in [Table 5](#).

Table 5. Intended use and maximum use level for the extension of use of the novel food

Food category	Maximum use level
Infant formula as defined under Regulation (EU) No 609/2013	1.2 g/L
Follow-on formula as defined under Regulation (EU) No 609/2013	1.2 g/L
Processed cereal-based foods for infants and young children and baby foods for infants and young children as defined under Regulation (EU) No 609/2013	0.7 g/L (for liquid food ready for use, marketed as such or reconstituted) 7.0 g/kg (products other than beverages)
Unflavoured pasteurised and unflavoured sterilised (including UHT) milk	1.0 g/L
Dairy analogues, non-dairy yogurts	1.0 g/L (beverages) 10.0 g/kg (products other than beverages)
Unflavoured fermented milk-based products Yogurts	1.0 g/L (beverages) 10 g/kg (products other than beverages)
Flavoured fermented milk-based products including heat-treated products	1.0 g/L (beverages) 10.0 g/kg (products other than beverages)
Cereal bars	30.0 g/kg
Breakfast cereals, ready to eat	40.0 g/kg
Milk based drinks and similar products intended for young children	1.2 g/L
Flavoured drinks Energy and sports drinks	1.0 g/L
Total diet replacement foods for weight control as defined under Regulation (EU) No 609/2013	2.0 g/L (liquid products) 30.0 g/kg (solid products e.g. bars)
Foods for special medical purposes as defined under Regulation (EU) No 609/2013 for infants and young children	In accordance with nutritional requirements
Foods for special medical purposes as defined under Regulation (EU) No 609/2013 excluding foods for infants and young children (under 3 years)	In accordance with nutritional requirements
Food Supplements * for the general population, excluding infants and young children	5.0 g/day
Food Supplements * for young children (aged 1 to 3 years)	1.2 g/day

* Food Supplements as defined in the Food Supplements (England) Regulations 2003, the Food Supplements (Wales) Regulations 2003 and the Food Supplements (Scotland) Regulations 2003

The anticipated intake for 3-FL in children up to the age of 16 weeks is estimated to be 312 mg/kg body weight/day for a 6.7 kg infant. The estimated intake was calculated from the use of 3-FL in infant formula

(1.2 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 high daily intake of 3-FL in breast-fed infants per kg/BW (see [Table 4](#)).

The estimated mean and 95th percentile intakes in infants were determined using data from the UK Diet and Nutrition Survey of Infants and Young Children (DNSIYC). The mean intake levels of the novel food were reported as 164 and 167 mg/kg BW/day for infants aged 4 – 6 months and 7 – 12 months, respectively. For 95th percentile consumers, the intake levels were reported as 360 and 350 mg/kg BW/day in infants aged 4 – 6 months and 7 – 12 months, respectively. These values do not exceed the estimated high daily intake of 3-FL in breast-fed infants per kg/BW (see [Table 4](#)).

An intake assessment using the summary statistics of consumption from the dietary surveys in the EFSA Comprehensive European Food Consumption Database was conducted by matching the intended conditions of use with the FoodEx2 categories. The estimated mean and high-level intakes of 3-FL from the intended conditions of use for each population group are presented in [Table 6](#).

Table 6. Estimated mean and 95th percentile intake levels for the novel food using the EFSA Comprehensive European Food Consumption on a body weight basis

Population Group	Mean 3-FL intake (mg/kg BW/day)	P95 3-FL intake * (mg/kg BW/day)
Young children	51 – 161	165 – 567
Other children	20 – 93	64 – 251
Adolescents	7 – 25	20 – 85
Adults	4 – 15	13 – 63
Elderly	4 – 14	6 – 48
Very elderly	5 – 12	7 – 47

BW = body weight; P95 = 95th percentile

Young children (12 – 35 months); other children (3 – 9 years); adolescents (10 – 17 years); adults (18 – 64 years); elderly (65 – 74 years); very elderly (75+ years)

* High level intake was calculated in DaDiet software using the HESS model based on total population intake and corresponds to an intake between the 95th and 97th percentiles for toddlers, between the 96th and 99th percentiles for the elderly, and between the 95th and 99th percentiles for other children, adolescents, adults, and the very elderly.

The highest mean and 95th percentile intakes of 3-FL on a body weight basis are reported in the young children population group, 161 mg/kg BW/day and 567 mg/kg BW/day, respectively. The 3-FL exposure levels in this population group are not unexpected 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

highest estimated 95th percentile intake value of 567 mg/kg BW/day does not exceed the estimated high daily intake of 3-FL in breast-fed infants per kg/BW (see [Table 4](#)).

Food supplements are intended to be used by young children at a maximum dose of 1.2 g/day, and by other children, adolescents, adults and the elderly at a maximum dose of 5.0 g/day. Food supplements are not intended to be used if other foods with the novel food are consumed on the same day.

The estimated intake for 3-FL from food supplements at the intended use levels for all population groups on a body weight basis is presented in [Table 7](#).

Table 7. Estimated daily intake of 3-FL from food supplements on a body weight basis

Population Group	Proposed use level (g/day)	Mean body weight (kg) *	Estimated intake (mg/kg BW/day)
Young children	1.2	11.9	101
Other children	5.0	23.1	216
Adolescents	5.0	43.4	115
Adults	5.0	73.9	68
Elderly	5.0	76.0	66
Very elderly	5.0	71.2	70

* EFSA Scientific Committee, 2012

Young children (12 – 35 months); other children (3 – 9 years); adolescents (10 – 17 years); adults (18 – 64 years); elderly (65 – 74 years); very elderly (75+ years)

BW = body weight

The highest estimated intake for 3-FL in food supplements of 216 mg/kg BW/day does not exceed the estimated high daily intake of 3-FL in breast-fed infants per kg/BW (see [Table 4](#)).

Risk managers may wish to consider precautionary labelling to ensure food and food supplements containing the novel food are not consumed on the same day. Additionally, food supplements containing 3-FL are not intended to be consumed by infants.

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

A pharmacokinetic study was conducted to assess the absorption of 3-FL during the 90-day repeat dose feeding study in rats (Mukerji, 2018 [unpublished]). Urine and blood samples were taken at day 80 for male rats and day 81 for female rats (10 rats per group). The rats were housed overnight in metabolic cages with access to food and water for at least 16 hours to collect urine. The levels of 3-FL were determined by collecting

serum samples at three different time points over an 8-hour period. The study was conducted according to GLP (Good Laboratory Practice) and the experimental design, results and analytical procedures are included in a separate technical report (Chan, 2019; Pitt et al., 2019 [unpublished]).

The 3-FL doses were calculated on the day of sampling and corresponded to approximately 2,300 and 4,700 mg/kg BW/day in males, and 3,200 and 5,900 mg/kg BW/day in females, for 5 and 10% diet, respectively. The results indicated low absorption of 3-FL in the serum, and the systemic exposure from dietary intake was proportional to dose (higher in female rats than male rats). Recovery of 3-FL in the urine was approximately 0.4 mole %. Quantitation of 3-FL in the serum and urine confirmed systemic exposure was negligible, and absorption of 3-FL was less than 1.0 % of daily dietary intake.

These results are supported by reports in the literature. Engfer *et al.* (2000) suggested that human milk oligosaccharides are not digested in the upper small intestine. Goehring *et al.* (2014) demonstrated that less than 5% of ingested 3-FL is absorbed intact. Rudloff *et al.* (1996); Rudloff & Kunz (2012); Obermeier *et al.* (1999); Chaturvedi *et al.* (2001); Dotz *et al.* (2014) all detected low quantities of 3-FL unchanged in the urine of breast-fed infants.

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 is not expected to pose a safety concern for infants or other age groups.

Human milk oligosaccharides are reported to stimulate the growth of probiotic bacteria by interfering with bacteria-host interactions and strongly influence the composition of the gut microflora. These observations indicate a prebiotic function for these oligosaccharides (Newburg et al., 2005; O'Hara & Shanahan, 2006; Zivkovic et al., 2010).

Thongaram *et al.* (2017) investigated the ability of different probiotics (*Bifidobacteria* and *Lactobacilli*) to ferment human oligosaccharides. Only *Bifidobacterium longum* ssp. *infantis* ATCC 15697 and *Bifidobacterium infantis* M-63 were able to ferment 3'-sialyllactose, 6'-sialyllactose, 2'-fucosyllactose, and 3-fucosyllactose.

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

2.9. Nutritional information

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 intended use levels is not expected to be nutritionally disadvantageous for consumers.

2.10. Toxicological information

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

2.10.1. Genotoxicity

In vitro genotoxicity testing of 3-FL was conducted under GLP conditions (OECD, 1998) and according to the OECD guidelines: *in vitro* bacterial reverse mutation test (OECD TG 471) and *in vitro* mammalian cell micronucleus test (OECD TG 487). This is the approach recommended by the UK Committee on Mutagenicity and in the guidance on the preparation and submission of an application for authorisation of a novel food in the context of assimilated Regulation (EU) 2015/2283.

The *in vitro* bacterial reverse mutation test (OECD, 1997) was conducted with the *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537, and the *Escherichia coli* strain WP2 uvrA (pKM101). A preliminary test (8 doses ranging from 33.3 to 5,000 µg/plate) and the main mutagenicity test (333, 667, 1,000, 3,333 and 5,000 µg/plate) were conducted with a plate incorporation method either in the presence or absence of S9 metabolic activation with the novel food (94.6% 3-FL) in aqueous solution. No substantial, reproducible or dose-related increases in revertant colony numbers over the controls were reported in any of the bacterial strains following exposure to the novel food at any concentration. Further, no appreciable toxicity or precipitation was observed following exposure to any dose of the novel food. Therefore, 3-FL is non-mutagenic at concentrations up to 5,000 µg 3-FL/plate, in the absence or presence of metabolic activation (Faranda, 2018 [unpublished]).

The *in vitro* mammalian cell micronucleus test (OECD, 2016a) was conducted by exposing Chinese hamster ovary (CHO) cells to the novel food (94.6% 3-FL) at 500, 1,000, 2,500, 3,500 and 5,000 µg 3-FL/mL, in the presence or absence of S9 metabolic activation, for 4 or 24 hours *in vitro*. No toxicity to the CHO cells or precipitation was observed and the percentage of micronuclei was not significantly increased in any of the test substance concentrations. All reported values were within the 95% historical negative control limits; however, evidence of a statistically

significant ($p \leq 0.05$, William's test) increasing concentration-related trend ($> 2,500 \mu\text{g}$ 3-FL/mL) in the 4-hour test with metabolic activation was noted. A further confirmatory assay was conducted with 3-FL concentrations ranging from 500 to 5,000 $\mu\text{g}/\text{mL}$. Similar results were obtained, and substantial cell toxicity ($55 \pm 5\%$ mitotic reduction) was observed at 1,000 and 5,000 $\mu\text{g}/\text{mL}$ (Kellum, 2018 [unpublished]). According to the relevant OECD TG 487 guidelines, these results were considered equivocal, so further genotoxicity investigations were conducted.

Additional *in vitro* and *in vivo* genotoxicity testing of 3-FL was conducted under GLP conditions and according to OECD guidelines: *in vitro* mammalian cell chromosomal aberration test (OECD TG 473) and *in vivo* mammalian erythrocyte micronucleus test (OECD TG 474).

The *in vitro* mammalian cell chromosomal aberration test (OECD, 2016c) was conducted by exposing human lymphocytes to the novel food (94.6% 3-FL) at 78.13, 156.25, 312.5, 625, 1,250, 2,500 and 5,000 μg 3-FL/mL, in the presence or absence of S9 metabolic activation, for 4 or 24 hours *in vitro*. No dose-dependent toxicity was observed, and 3-FL did not induce any statistically significant increases in the frequency of structural or numerical aberrations. Therefore, 3-FL was not clastogenic at concentrations up to 5,000 μg 3-FL/mL, in the absence or presence of S9 metabolic activation (Lacey, 2019 [unpublished]).

The *in vivo* mammalian erythrocyte micronucleus test (OECD, 2016b) evaluated the ability of the 3-FL to induce micronuclei in the bone marrow of Crl:CD1(ICR) mice. Five male and five female (seven males and seven females at the highest dose) mice were administered a single dose of novel food (94.6% 3-FL) at 500, 1,000 and 2,000 mg 3-FL/kg BW by oral gavage. Peripheral blood reticulocytes were analysed after 48 and 72 hours. No statistically significant or biologically relevant increases in the frequency of micro-nucleated reticulocytes in peripheral blood samples were reported (Myhre, 2018 [unpublished]). Confirmation of systemic exposure to 3-FL was demonstrated by analysing pooled plasma collected 4 hours after a further four female and four male mice were administered a single dose of 500 mg/kg of 3-FL (see ADME Section 3.8).

The results from these *in vitro* and *in vivo* studies support the conclusion that 3-FL is not genotoxic.

2.10.2. Acute and sub-acute toxicity

An acute toxicity: up and down procedure conducted under GLP and according to OECD TG 425 guidelines (Fallers, 2018 [unpublished]) was provided. The FSA and FSS do not consider acute toxicity studies relevant for the safety assessment of novel foods.

A preliminary 6-day piglet study (Bond, 2019b [unpublished]) using a batch of novel food containing 95% 3-FL was conducted. Two male and two female piglets were given a dose of approximately 975 mg/kg BW/day of 3-FL in milk replacer offered via dish feeding. No deaths and no adverse effects on clinical observations, body weight, food consumption and food efficiency, clinical pathology or macroscopic observations in any of the animals were reported.

In the main 21-day study (Bond, 2019a [unpublished]), conducted under GLP conditions, six male and six female neonatal piglets were given the same batch of novel food at 1 or 2 g/L of 3-FL in 500 mL/kg BW (in milk replacer six times a day). This corresponded to an approximate average dose of 450 and 900 mg/kg BW/day at the two dose levels of 3-FL. An additional group were given fructo-oligosaccharide at 2 g/L as a comparator.

No deaths or clinical signs were noted. A slight non-statistically significant reduction in food consumption and body weight was observed for piglets receiving the highest dose of 3-FL, mainly in males. A slight variation in the red blood cell parameters was recorded (statistical significance for the mean corpuscular volume and haemoglobin concentration) at 2 g 3-FL/L in males on Day 22. A small statistically significant decrease in alkaline phosphatase was noted, mainly at the dose of 2 g/L for both 3-FL and fructo-oligosaccharide. At the highest 3-FL dose in males, a small decrease in globulins was also recorded. No other variations in chemical chemistry and coagulation parameters were noted. No gross pathology or histological alterations were observed.

In summary, there were no test article-related findings and the administered doses appeared to be well tolerated, but there were signs of reduced food intake at the highest dose of 3-FL in male piglets.

2.10.3. Sub-chronic toxicity

A repeat dose 90-day oral toxicity study in rodents (Mukerji, 2018 [unpublished]) was conducted under GLP conditions according to OECD TG 408 guidelines (OECD, 2018) 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.

In this 90-day feeding study, each group consisted of 10 female and 10 male rats which were fed 0 (control – vehicle only [water]), 5% w/w or 10% w/w of 3-FL by oral gavage. A reference control group consisting of the same number of animals was fed 10% w/w fructo-oligosaccharides. Blood

and urine samples were collected from all animals during week 12 of the study to determine the plasma and urine levels of 3-FL (see ADME Section 3.8).

No deaths or test substance-related effects on clinical observations, ocular changes, or differences in body weight between the test groups were reported. A statistically significant reduction in body weight gain in the 5% and 10% male 3-FL groups compared to the control during one weekly interval was reported. Considering no other differences were observed, this finding was considered unrelated to the test substance.

There was a statistically significant reduction in food consumption reported in one weekly interval and a statistically significant increase in two weekly intervals in the 5% female 3-FL group compared to the control. In the 10% female 3-FL group, a statistically significant reduction in one weekly interval compared to the control was also reported. In addition, a statistically significant reduction in food efficiency in the 5% male 3-FL group compared to the control was noted. These findings showed no concentration dependent relationship and were considered unrelated to the test substance.

There were no statistically significant changes in coagulation parameters, clinical chemistry, urinalysis, organ weights or clinical pathology. Statistically significant higher mean cell volume and mean cell haemoglobin values were reported in the male 5% 3-FL male group. These findings did not occur in a dose-related manner and were not associated with changes in any other haematology parameter. Therefore, these observations were considered unrelated to test substance.

No dose related abnormalities were noted during the necropsy or histopathological evaluation. Therefore, the FSA and FSS consider that the no observable adverse effect level (NOAEL) for 3-FL is the high-dose of 10% w/w of 3-FL (corresponding to 5,975 and 7,270 mg/kg BW/day for males and females, respectively).

2.10.4. Human studies

No human clinical trials were conducted with the novel food.

2.11. Allergenicity

The protein content of the novel food is low as indicated by the compositional analysis data ([Table 2](#)) and the specification of the novel food ([Table 3](#)).

An analysis of the proteins expressed by the genetically modified production micro-organism using the AllerTOP algorithm indicates that none of these were predicted to be allergenic.

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

3. Discussion

The novel food is a human identical milk trisaccharide containing $\geq 90.0\%$ dry weight of 3-FL, which is manufactured by microbial fermentation using a genetically modified strain of *Escherichia coli* K-12.

3-FL is intended to be used in dairy products and analogues, cereals, bakery wares, foods for special groups, beverages, and food supplements. Foods containing the novel food are intended for the general population. Food supplements are intended to be consumed by all population groups, except for infants. Food supplements are not intended to be used if other foods with added 3-FL or breast milk are consumed in the same day.

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.

In the repeat dose 90-day oral toxicity study in rodents, the NOAEL for 3-FL was 5,975 mg/kg BW/day, the highest dose tested. When this NOAEL is compared with the highest estimated exposure in each population group, the margins of exposure range from 11 to 127. 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.

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.

The use level of 3-FL in food supplements (1.2 g/day for young children, and 5 g/day for all other population 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 other children, adolescents, adults and the elderly would not be expected to exceed the intake levels of 3-FL in breastfed infants on a body weight basis.

Risk managers may wish to consider precautionary labelling to ensure food and food supplements containing the novel food are not consumed on the same day. Additionally, food supplements containing 3-FL are not intended to be consumed by infants.

4. Conclusions

The FSA and FSS have undertaken the assessment of the novel food, which is composed mainly of 3-FL, and concluded that the novel food is safe under the proposed conditions of use and does not pose a safety risk to human health. The anticipated intake level and the proposed use in food and food supplements was not considered to be nutritionally disadvantageous.

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, and stability.
- bacterial reverse mutation test (Faranda, 2018 [unpublished]);
- *in vitro* micronucleus test (Kellum, 2018 [unpublished]);
- mammalian cell chromosomal aberration test in human lymphocytes (Lacey, 2019 [unpublished]);
- mammalian erythrocyte micronucleus test (Myhre, 2018 [unpublished]);
- pharmacokinetic study (Chan, 2019 [unpublished]);
- 90-day repeat dose feeding study with the novel food (Mukerji, 2018 [unpublished]).

Abbreviations

Abbreviation	Definition
2'-FL	2'-fucosyllactose
3-FL	3-fucosyllactose
6'-SL	6'-sialyllactose
ADME	Absorption, Distribution, Metabolism and Excretion
BCCM/LMG	Belgian Co-ordinated Collections of Micro-organisms (Laboratory of Microbiology, Ghent University)

Abbreviation	Definition
BW	body weight
CAS	Chemical Abstracts Service
CFU	Colony forming unit
DIN	German Institute for Standardization
DM	Dry matter
EC	European Commission
EFSA	European Food Safety Agency
EU	European Union
EU/1,000 mg	Endotoxin units per 1000 mg
Eur. Ph.	European Pharmacopoeia
FDA	Food and Drug Administration
GLP	Good Laboratory Practice
GMM	Genetically modified microorganism
GMO	Genetically modified organism
HACCP	Hazards Analysis and Critical Control Points
HILIC	Hydrophilic liquid interaction chromatography
HMBC	Heteronuclear multiple bond correlation
HMO	Human milk oligosaccharide
HSQC	Heteronuclear single quantum correlation
ISO	International Organisation for Standardization
IUPAC	International Union of Pure and Applied Chemistry
LNT	Lacto-N-tetraose
LOD	Limit of detection
LOQ	Limit of quantification
NMKL	Nordic Committee on Food Analysis
NMR	Nuclear magnetic resonance
NOAEL	No observable adverse effect level
OECD	Organisation for Economic Co-operation and Development
QPS	Qualified Presumption of Safety
RH	Relative humidity
RI	Refractive index
TS	Technical standard
UHT	Ultra-high temperature
USP	United States Pharmacopoeia

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