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Supporting document 1

Risk and technical assessment – Application A1340

Application A1340 - 2′-FL from GM *Escherichia coli* BL21 (gene donor: *Akkermansia muciniphila)* for use as a nutritive substance in infant formula products

Executive summary

Food Standards Australia New Zealand (FSANZ) has assessed an application from Suzhou Yixi Biotech Co., Ltd. to amend the Australia New Zealand Food Standards Code (the Code) to permit a new source organism for the production of 2′-fucosyllactose (2′-FL). The applicant's 2′-FL is produced by microbial fermentation using a strain of genetically modified (GM) *Escherichia coli* BL21 containing the gene for alpha-1,2-fucosyltransferase from *Akkermansia muciniphila.*

The Code already permits 2′-FL from several source organisms to be used as a nutritive substance in infant formula products. FSANZ has determined that the applicant's 2′-FL is chemically, structurally and functionally identical to 2′-FL naturally present in human milk. No public health or safety concerns were identified, and the established health benefits remain unchanged.

Analytical data confirm that the applicant's 2′-FL is chemically and structurally identical to the naturally occurring substance in human milk, similar to 2′-FL previously assessed and permitted by FSANZ. There is an appropriate specification for 2′-FL from *E. coli* BL21 in the Code. The applicant's 2′-FL is stable under ambient storage conditions.

FSANZ's previous assessments found no safety concerns for 2′-FL at concentrations up to 2.4 g/L in infant formula products. Newly available data support this conclusion. No adverse effects were observed in a 90-day oral toxicity study in rats and a prenatal developmental toxicity study in rats. Human clinical studies further confirm that infant formula products containing 2′-FL are safe and well tolerated. 2′-FL was not genotoxic in vitro or in vivo.

FSANZ's safety assessment did not identify any public health and safety concerns associated with the use of the applicant's GM strain of *E. coli* BL21 as a production organism for 2′-FL. Characterisation of the production strain confirmed that all introduced genes were genetically stable and functional.

Based on previous benefit assessments and given that the 2′-FL is chemically, structurally and functionally identical, with no change requested to the maximum amount in infant formula products, the associated health benefits from the use of 2′-FL remain unchanged. These benefits are: (1) an anti-pathogenic effect; (2) immunomodulation; and (3) development of the gut microbiome by supporting growth of Bifidobacteria spp.

FSANZ has previously concluded that the addition of 2′-FL in infant formula products at levels typically found in human milk does not pose a risk to normal growth. One new study was reviewed for the current assessment, however due to a number of study limitations, this was not included in the body of evidence. Therefore, FSANZ maintains its previous conclusion.

Based on the available data, there are no public health and safety concerns associated with the addition of 2′-FL produced from the new source organism to infant formula products under the proposed conditions.

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1 Introduction

Food Standards Australia New Zealand (FSANZ) received an application from Suzhou Yixi Biotech Co., Ltd. to amend the Australia New Zealand Food Standards Code (the Code) to permit a new source organism for the production of 2′-fucosyllactose (2′-FL). The applicant's 2′-FL is produced by microbial fermentation using a strain of *Escherichia coli* BL21 containing the gene for alpha-1,2-fucosyltransferase from *Akkermansia muciniphila*.

The Code already permits 2′-FL from several source organisms to be used as a nutritive substance in infant formula products. The maximum amount of 2′-FL in infant formula products is 96 mg/100 kJ, equivalent to 2.4 g/L. The purpose of the present assessment is therefore to assess the safety of 2′-FL produced by the new production strain.

2 Food technology assessment

The objective of the food technology assessment is to determine whether the 2′-FL to be added to infant formula products from a new microbial source is identical to that present in human milk. The assessment also considered the manufacturing process, stability, specification and the analytical methods of analysis used to quantify and characterise 2′-FL.

FSANZ has assessed recent applications requesting permissions for human-identical milk oligosaccharides (HiMO) for use in infant formula products The information in this section has built on the assessment of those applications, i.e. A1155, A1190, A1233, A1251, A1265, A1277, A1283, A1308, A1324 (FSANZ 2020, FSANZ 2021, FSANZ 2022a, FSANZ 2022b, FSANZ 2023a, FSANZ 2023b, FSANZ 2024, FSANZ 2025a, FSANZ 2025b).

2.1 Chemical and physical properties

2′-FL is part of the human milk oligosaccharide (HMO) fraction found in human milk. It is made up of l-fucose, D-galactose, and D-glucose. Its structure includes L-fucose connected to D-lactose by an alpha (1→2) glycosidic bond (see Figure 1).

It is a white to off-white amorphous powder or agglomerate and is readily soluble in aqueous solutions but has limited solubility in organic solvents.

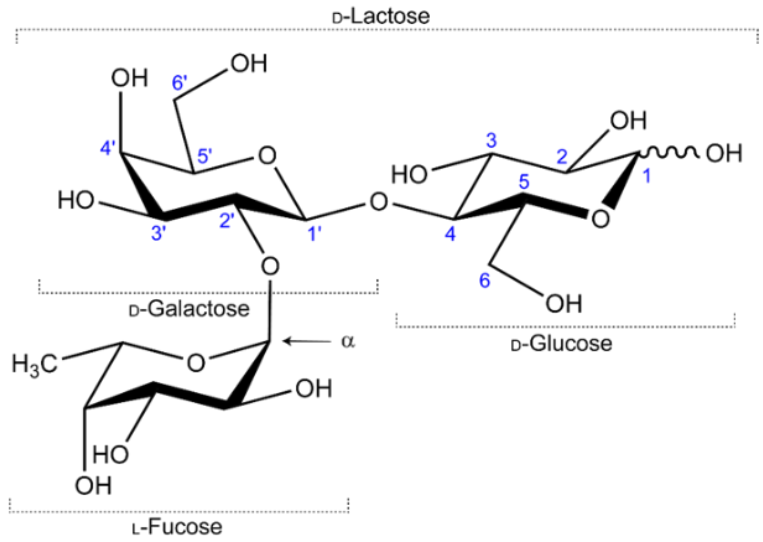

Figure 1: Molecular structure of 2'-FL

2.1.1 Equivalence to human milk

The application included analytical data provided as Confidential Commercial Information (CCI) to demonstrate that 2′-FL obtained from microbial fermentation is chemically and structurally identical to 2′-FL naturally present in human milk. The analytical methods used to determine the chemical structure were nuclear magnetic resonance (NMR) spectroscopy, including hydrogen spectrum NMR (H-NMR), carbon spectrum NMR (C-NMR), DEPT spectrum and two-dimensional spectra. Liquid-phase mass spectrometry (LC-MS) and high-resolution mass spectrometry (HR-MS) were used to confirm the molecular structure.

The NMR and MS results confirm the 2′-FL obtained from microbial fermentation is chemically and structurally identical to that occurring naturally in human milk. The chemical name and properties of the applicant's 2′-FL are provided in Table 1.

Table 1: Chemical name and properties of 2'-FL

Property	2′-FL
Common name	2′-fucosyllactose
IUPAC[1] name	α-L-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→4)-D-glucopyranose
Alternative common names	2′-O-Fucosyllactose 2′-Fucosidolactose 2-Fucosyl-D-Lactose 2′-O-L-Fucosyl-D-lactose Fucosyl-α-1,2-galactosyl-β-1,4-glucose Fuc-α-(1→2)-Gal-β-(1→4)-Glc
CAS[2] registry number	41263-94-9
Chemical formula	$C_{18}H_{32}O_{15}$
Molecular weight	488.44 g/mol

[1] The International Union of Pure and Applied Chemistry
[2] Chemical Abstract Service

2.1.2 Stability of 2′-FL under intended conditions of use

Analytical data for the applicant's 2′-FL showed stability for at least 12 months under ambient conditions (25°C, 60% RH) and 15 months under accelerated conditions (37°C, 75% RH). The applicant states that the results support stability under ambient conditions for up to 2 years.

Analytical data for the applicant's 2′-FL when added to infant formula milk powder indicated stability for 6 months under accelerated conditions (40°C, 75% RH).

2.2 Manufacturing processes

The applicant's 2′-FL is produced by microbial fermentation of a GM strain of *E. coli* BL21 as described in section 3.1. There are two main steps in the production process: fermentation and purification. Details of the manufacturing flow and processes, raw materials and food safety management systems were provided as CCI.

2.3 Specification

Section 1.1.1—15 of the Code requires that a substance used as a nutritive substance must meet any relevant identity and purity specification in Schedule 3. There is a specification for 2′-FL from *E. coli* BL21 in S3—45. This specification was included in the Code in association with the approval of 2′-FL sourced from *E. coli* BL21 containing the gene for alpha-1,2-fucosyltransferase from *E. coli* O126 for use as a nutritive substance (approved under Application A1190 – 2′-FL in infant formula and other products). The specification includes parameters for the identity of 2′-FL and impurities.

The applicant provided a proposed specification for its 2′-FL that aligns with the existing specification for 2′-FL from *E. coli* BL21 in S3—45 (see Table 2) with the exception of three parameters.

Firstly, the applicant proposed that the parameter for 'GMO detection – not detected' is omitted (see section 2.3.1).

The applicant also requested an increase to the 2′–FL purity in the relevant specification under subsection S3—45(e) from 90.0% to 94.0% because their 2′-FL is more highly purified. In association with this, they requested removal of the limit of not more than 3% for fucosyl-galactose, given this would be no longer applicable with a higher 2′-FL content. Since 2′-FL is adequately identified in S3—45 and no safety or technological concerns have been identified regarding the current limits for 2′-FL and fucosyl-galactose, FSANZ is proposing that these limits remain unchanged (see Table 2).

2.3.1 Impurities

The applicant's product contains a minimum of 94% 2′-FL and an absence of fucosyl-galactose. There are smaller amounts of other carbohydrates present, including D-lactose, 3-Fucosyllactose, difucosyl-D-lactose, D-glucose and D-galactose. The applicant submitted analytical data for 3 batches of their 2′-FL which met the currently approved specification for 2′-FL in S3—45. Additionally, separate testing confirmed DNA or proteins from the production strain were absent in the final product.

The current specification for 2′-FL from *E. coli* BL21 in S3—45 includes a requirement for 'GMO detection – not detected'. This criterion is inconsistent with other 2′-FL specifications in the Code (S3—40, S3—51, S3—54(A)), and, given that residual DNA and proteins from the production strain have been shown to be absent, is considered redundant. This redundancy also applies to the existing permission approved under Application A1190. FSANZ therefore proposes removing the GMO non-detection requirement from the specification, to ensure consistency and regulatory clarity between permissions.

Table 2: Proposed specification for 2'-FL from E. coli BL21 (based on current specification in S3—45)

Parameter	Specification
Chemical name	α-L-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→4)-D-glucopyranose
Chemical formula	$C_{18}H_{32}O_{15}$
CAS number	41263-94-9
Description	White to off-white ivory powder, or a colourless to slightly yellow liquid

2′-FL	not less than 90.0%
D-lactose	not more than 5.0%
L-fucose	not more than 3.0%
3-fucosyllactulose	not more than 5.0%
difucosyllactose	not more than 5.0%
fucosyl-galactose	not more than 3.0%
glucose	not more than 3.0%
galactose	not more than 3.0%
water	not more than 9.0% for powder, n/a for liquid
solids	45% w/v (± 5%) dry matter in water, not applicable for powder
ash, sulphated	not more than 0.5%
residual proteins	not more than 0.01%
lead	not more than 0.02 mg/kg
arsenic	not more than 0.2 mg/kg
cadmium	not more than 0.1 mg/kg
mercury	not more than 0.5 mg/kg
Microbiological	
Salmonella	absent in 100 g for powder, absent in 200 mL for liquid
total plate count	not more than 10000 cfu/g for powder, not more than 5000 cfu/g for liquid
Coliforms/enterobacteriaceae	absent in 11 g for powder, absent in 22 mL for liquid
yeasts and moulds	not more than 100 cfu/g for powder, not more than 50 cfu/g for liquid
aflatoxin M1	not more than 0.025 ug/kg
endotoxins	not more than 10 EU/mg

2.4 Analytical methods for detection

Internal methods and Chinese standard methods from the Chinese Pharmacopoeia and Guo Biao (GB) (Chinese national food safety standards) have been utilised to detect and quantify 2′FL and other parameters in the proposed specification. This includes for other carbohydrates, water, residual proteins, heavy metals and microbiological components.

2.5 Food technology conclusion

The applicant's 2′-FL, produced by a microbial fermentation method of production, is chemically and structurally identical to the naturally occurring substance present in human milk. It is also chemically and structurally identical to 2′-FL previously assessed and permitted by FSANZ.

There is an appropriate specification in S3—45 for 2′-FL from *E. coli* BL21. FSANZ is proposing minor amendments to that specification for clarity and consistency with other specifications in Schedule 3.

Stability studies of the applicant's 2′-FL concluded that the nutritive substance as a powder is stable for 12 months when stored at ambient conditions (25°C and 60% RH) and up to 15 months under accelerated conditions (40°C and 75% RH). The applicant's 2′-FL when added to powdered infant formula is stable for 6 months under accelerated conditions (40°C, 75% RH).

3 Safety assessment

Some information relevant to this section is CCI, so full details cannot be provided in this public report.

3.1 GM production strain assessment

3.1.1 Host organism

The host organism used by the applicant is *Escherichia coli* BL21 (DE3). This organism is a direct derivative of *E. coli* BL21 with minimal, well characterised divergence. Due to this minimal divergence, FSANZ's microbiological assessment will determine the safety of the *E. coli* BL21(DE3) at the strain level of BL21.

Escherichia coli is a facultative anaerobic, gram-negative, rod-shaped bacteria found in the gut of mammals (Guerra et al. 2019). *E. coli* strains can be pathogenic to humans causing a wide range of diseases, some of which can be fatal (Guerra et al. 2019, Mir and Kudva 2019). However, there are strains of *E. coli*, termed safe strains, that are used in research and industry specifically because of their inability to cause disease in humans (Bauer et al. 2008). *E. coli* BL21 is one of these safe strains. *E. coli* BL21 lacks well-recognised pathogenic mechanisms (Chart et al. 2000). As such it is classified as non-pathogenic and is categorised as a Biosafety Level 1 organism (Pinske et al. 2011). Along with other safe strains of *E. coli*, including strains K-12, B, C, and their derivatives, BL21 is designated as a Risk Group 1 organism (Bauer et al. 2008).

Escherichia coli BL21 is widely used in biotechnology, molecular biology, and industrial applications (Pinske et al. 2011, Kim et al. 2017). Originally developed for its utility as an expression host, BL21 has a long history of safe use in research and production of recombinant proteins and other bioproducts. Because of its extensive industrial use, BL21 has previously been assessed by regulatory agencies, including the U.S. Food and Drug Administration (FDA), the European Food Safety Authority (EFSA) and FSANZ (in applications A1190 and A1318), for use in industrial processes under Good Manufacturing Practices (GMP). The use of BL21 as the production organism of food ingredients has been concluded to be 'generally recognised as safe' (GRAS) numerous times over the past decade (e.g. GRNs 485, 571, 876, 921, 922, 923, 925, 1015, and 1016).

The *E. coli* BL21 production strain assessed here has been genetically modified to contain a gene from *Akkermansia muciniphila* expressing the α-1,2-fucosyltransferase enzyme for the purpose of 2′-FL production, see section 3.1.3 (Characterisation of the GM production organism) for more detail. The taxonomic identity of the production strain was confirmed by CCI data provided by the applicant. Furthermore, batch analyses demonstrate that the final product is absent of residual DNA and protein from the production organism.

Overall, no public health and safety concerns were identified for the use of *E. coli* BL21 as a production organism.

3.1.2 Gene donor organisms

The gene donor organisms are *E. coli* K12 and *A. muciniphila*. All inserted genes were synthesised chemically prior to insertion. This avoids the possibility of any extraneous DNA from the gene donor organisms being inadvertently transferred to the production strain.

3.1.3 Characterisation of the GM production organism

3.1.3.1 Development of the GM production strain

The production strain *E. coli* EC102 was developed by modifying the host *E. coli* BL21 genome in three sequential steps as follows:

1. Partial knockout of the *lacZ* gene, encoding β-galactosidase;
2. Substitution of the *wca*KLM gene, which is involved in colanic acid biosynthesis, with the *FRS-GRS-CRS-BRS* gene cluster from *E. coli* K12;
3. Substitution of the *wca*J gene, encoding UDP-glucose lipid carrier transferase, with the *AKTRS* gene from *A. muciniphila*.

The *FRS-GRS-CRS-BRS* gene cluster from *E. coli* K12 encodes:

- GDP-L-fucose synthase;
- GDP-D-mannose-4,6-dehydratase;
- alpha-D-mannose phosphate guanylyltransferase; and
- phosphomannomutase.

The *AKTRS* gene from *A. muciniphila* encodes alpha-1,2-fucosyltransferase.

Together, the inserted heterologous genes direct the biosynthesis of 2′-FL.

Each of the inserted genes were introduced into the genome of the host strain using CRISPR/Cas9 editing. Each target gene and its corresponding single guide RNA (sgRNA) sequence were cloned into the plasmid vector pTargetF. Following editing, the plasmid vectors were removed, resulting in the final production strain EC102.

3.1.3.2 Characterisation of introduced DNA

Polymerase chain reaction (PCR) was used to verify the presence of the inserted DNA in the production strain. These analyses confirmed the presence of the intended genomic insertions in the production strain.

3.1.3.3 Genetic stability and inheritance of the introduced DNA

The PCR analyses that were used to demonstrate the presence of the inserted DNA in the production strain also showed that these insertions were stable over 8 successive generations. Transcription levels from the exogenous genes were measured by reverse transcription quantitative PCR (rt-qPCR) and were shown to be stable over 8 generations.

Data was also provided showing the growth rate and production of 2′-FL by the production strain was stable over 8 generations, providing further indirect evidence for the stability of the inserted DNA.

3.1.4 Conclusion

FSANZ's microbiological risk assessment did not identify any public health and safety concerns associated with the use of *E. coli* BL21 as a production organism for 2′-FL.

Characterisation of the GM production strain confirmed all introduced genes were inserted as expected and were genetically stable and functional.

Based on the data provided, no safety concerns were identified in the assessment of the 2′-FL production strain.

3.2 Toxicology assessment

3.2.1 Previous FSANZ safety assessments of 2′-FL

A range of toxicological and human clinical studies of 2′-FL have previously been reviewed by FSANZ as part of multiple applications, as listed at the following link: Current status of genetically modified foods applications | Food Standards Australia New Zealand.

2′-FL is structurally and chemically identical to the form naturally present in human milk. As such, no differences in pharmacokinetics or safety between the naturally occurring and manufactured forms of 2′-FL is expected. Intestinal absorption is limited, and a significant proportion of 2′-FL reaches the large intestine where it is fermented by the microbiota or excreted unchanged in the faeces. 2′-FL is not genotoxic and does not produce adverse effects in short-term oral toxicity studies, including studies using neonatal animal models. In human clinical studies, consumption of infant formula products containing 2′-FL was safe and well tolerated.

3.2.2 Newly available data

The applicant submitted several proprietary toxicological studies with their 2′-FL preparation which were reviewed in the present assessment:

- Acute oral toxicity study in mice
- 90-day oral toxicity study in rats
- Bacterial reverse mutation assay
- In vivo micronucleus test in mice
- In vivo spermatogonial chromosome aberration assay in mice
- Developmental toxicity study in rats

All studies were performed by China Metrology Accreditation (CMA)-certified laboratories, following the evaluation procedures and methods of the National Food Safety Standard GB 15193 (GB 15193. National Food Safety Standard – General Rules for Toxicological Assessment of Food. Standardization Administration of the People's Republic of China, Beijing).

The applicant also conducted literature searches to identify new toxicological and human clinical studies of 2′-FL published between January 2022 and June 2025.

No toxicological studies that have not previously been reviewed by FSANZ were identified. Seven human studies were identified, two of which were previously evaluated by FSANZ (Jochum et al 2023, reviewed in application A1308, and Lazarini et al 2025, reviewed in application A1334). Four additional studies were not considered relevant for the present assessment as they did not report measures of safety such as adverse events. The remaining study was included in the evaluation.

3.2.2.1 Toxicological studies with the applicant's 2'-FL

Acute oral toxicity study in rats (CCI) Regulatory status: Conducted in a CMA-accredited laboratory, in accordance with National Food Safety Standard GB 15193.3-2014

In an acute oral toxicity study, groups of 10 male and 10 female ICR mice were administered

a single dose of 10,000 mg/kg bw 2′-FL. Animals were monitored for 14 days following treatment then underwent gross necropsy. All animals survived to the end of the study and no clinical signs of toxicity were observed. No abnormalities were found in the gross pathology examinations. The LD_{50} of 2′-FL in this study was > 10,000 mg/kg bw.

90-day oral toxicity study in rats (CCI) Regulatory status: Conducted in a CMA-accredited laboratory, in accordance with National Food Safety Standard GB 15193.13-2015

In a 90-day oral toxicity study, 2′-FL was administered in the diet to SD rats (10/sex/group) at concentrations of 0, 2.5%, 5.0% or 10.0% (equivalent to 0, 2000, 4000 or 8000 mg/kg bw/day). Additional mid-term satellite control and high dose groups (5/sex/group) were treated for 45 days then necropsied the following day. Clinical signs, body weight, food consumption and food efficiency were recorded throughout the study. Ophthalmic examinations were conducted on the satellite animals and those in the main study control and high dose groups. Haematology, clinical chemistry and urinalysis examinations were performed at 45 days (mid-term satellite groups) and at the end of the 90-day treatment period. All animals underwent gross pathological examination at termination and organ weights were recorded. Histopathological examinations were performed on tissues from animals in the satellite and main study control and high dose groups.

All animals survived to the end of the study. There were no treatment-related adverse effects on any of the test parameters. The no observed adverse effect level (NOAEL) in this study was 10.0% (equivalent to 8000 mg/kg bw/day), the highest dose tested.

Developmental toxicity study in rats (CCI) Regulatory status: Conducted in a CMA-accredited laboratory, in accordance with National Food Safety Standard GB 15193.14-2015

In a pre-natal developmental toxicity study, mated female SD rats were given 0 (n=19), 2000 (n=18), 4000 (n=18) or 8000 (n=17) mg/kg bw 2′-FL by oral gavage on gestation days (GD) 6–15. Water was used as the vehicle control. Clinical signs were monitored daily and body weights were recorded periodically. On GD 20 rats were killed and the uterine contents were examined. After gross examination of fetuses approximately half of each litter were prepared for skeletal examination while the other half were prepared for visceral examination.

No mortality or clinical signs of toxicity were observed in maternal animals. There were no treatment-related effects on body weight, gestational weight gain, weight of uterus and fetus and net weight gain of pregnant rats. There were no differences in the number of corpus lutea, implantations, live fetuses, absorbed fetuses and dead fetuses in the treated groups compared with control. There were no differences in length and body weight of fetuses. No treatment-related changes in the incidence of appearance, visceral and skeletal malformations were observed.

The NOAEL for maternal toxicity and for fetal toxicity was 8000 mg/kg bw/day, the highest dose tested.

3.2.2.2 Genotoxicity studies with the applicant's 2'-FL

Several genotoxicity studies with the applicant's 2′-FL were submitted. These studies were performed in accordance with Chinese testing requirements, and were generally consistent with OECD Test Guidelines with some deviations as noted below. Appropriate positive controls were included in these studies and produced the expected responses.

No evidence of mutagenicity, clastogenicity or aneugenicity were observed in these studies (Table 3).

Table 3: Genotoxicity studies of 2'-FL

Test[1]	Test object	Concentration	Results
Bacterial reverse mutation test (GB 15193.4-2014; generally equivalent to OECD TG 471)	Salmonella typhimurium TA97a, TA98, TA100, TA102 and TA1535	Test 1: 0, 50, 158.1, 500, 1581 or 5000 µg/plate Test 2: 0, 80, 40, 200, 1000 or 5000 µg/plate	Negative ± S9
In vivo mammalian erythrocyte micronucleus test (GB 15193.5-2014; generally equivalent to OECD TG 474)	ICR mouse bone marrow	0, 2500, 5000 or 10,000 mg/kg bw	Negative[2]
In vivo mammalian spermatogonial chromosomal aberration test (GB 159193-8-2014; generally equivalent to OECD TG 483)	ICR mouse spermatogonia	0, 2500, 5000 or 10,000 mg/kg bw	Negative[3]

[1] Study references are CCI.
[2] Study had several deviations from OECD Test Guideline 474: Bone marrow was collected 6 hours after the last treatment rather than the recommended 18–24 hours. Two thousand polychromatic erythrocytes per animal were scored for micronuclei, and 200 erythrocytes were scored for the proportion of immature erythrocytes, rather than the recommended 4000 and 500, respectively. However, positive controls produced the expected response. Bone marrow exposure was not confirmed in this study.
[3] One hundred metaphases from each animal underwent chromosome analysis, rather than the 200 recommended by OECD Test Guideline 483. However, positive controls produced the expected response. Systemic exposure was not confirmed in this study.

3.2.2.3 Human studies of 2'-FL

One study not previously reviewed by FSANZ and considered relevant to the assessment was identified in the applicant's literature search.

In a randomized, controlled, single-blinded (participants) crossover study, 82 Thai children aged 8–14 months were enrolled to consume a single serving of follow up formula containing isotopically labelled ferrous sulfate (2.2 mg iron) with 400 mg/100 mL, galacto-oligosaccharide (GOS) and *L. reuteri* DSM 17938, follow up formula containing 2′-FL (100 mg/100 mL), and follow up formula without GOS/*L. reuteri* or 2′-FL. The three formulas were consumed by participants in random order and a 3-day washout period took place between the interventions. Adverse events and serious adverse events were assessed by the study paediatrician during periodic visits to the study site. Seasonal influenza accounted for ~75% (n=33) of the total adverse events reported during the trial, followed by diarrhea (n=6) and other causes (n=6) such as insect bites, rashes, and skin injuries due to falling. Serious adverse events occurred in 2 cases due to gastroenteritis and herpangina. No adverse events were attributed to consumption of 2′-FL (Scheuchzer et al 2024).

3.2.3 Safety assessments by other agencies

As noted in previous FSANZ assessments, the European Food Safety Authority (EFSA) has

assessed 2′-FL from multiple sources as a novel food, including for addition to infant formula and follow-on formula. EFSA has concluded that 2′-FL is safe for its intended uses.

Health Canada has also completed novel food safety assessments for 2′-FL from a variety of sources. These reviews have concluded that 2′-FL does not pose a risk to human health when used as an ingredient in infant formulas and toddler formulas.

The applicant has indicated that a generally recognised as safe (GRAS) notification has been submitted to the US Food and Drug Administration (US FDA) and is currently under review.

3.2.4 Summary of the toxicology assessment

Based on previous FSANZ assessments of 2′-FL and the toxicological assessment in the present application, there are no public health and safety concerns associated with 2′-FL produced from the new source organism that is the subject of this application.

3.3 Microbiological assessment

FSANZ has completed assessments on microbiological risks and health benefits for previous applications concerning the production and addition of 2′-FL to infant formula products: A1155, A1190, A1233, A1251, A1265, A1277, A1283 and A1308 (FSANZ 2020, FSANZ 2021, FSANZ 2022a, FSANZ 2022b, FSANZ 2023a, FSANZ 2023b, FSANZ 2024, FSANZ 2025).

Based on these previous microbiological assessments and considering the identical chemical structure and functionality of the substance, and that the applicant has not requested any change in the maximum permitted amount of 2′-FL added to infant formula products, FSANZ has concluded that there are no microbiological public health and safety concerns. The associated health benefits from the use of 2′-FL as a nutritive substance in infant formula products remain unchanged. These include:

- An anti-pathogenic effect
- Immunomodulation
- Development of the gut microbiome through support of *Bifidobacteria* spp. growth.

3.4 Nutrition assessment

3.4.1 Objective of the nutrition assessment

The objective of the nutrition risk assessment is to determine the effect (if any) of the addition of 2′-FL to infant formula products on infant growth. The Code permits 2′-FL produced by several source organisms (as described in Section 1 above) to be used as a nutritive substance in infant formula products at a maximum amount of 96 mg/100 kJ, equivalent to 2.4 g/L. The applicant has requested permission for the use of 2′-FL from a genetically modified strain of Escherichia coli BL21(DE3) encoded with the gene for α-1,2-fucosyltransferase but has not requested a change to the permitted amount.

3.4.2 Previous FSANZ assessments of 2′-FL

FSANZ has previously conducted 9 assessments on the effect of the addition of 2′-FL to infant formula products on infant growth: A1155, A1190, A1233, A1251, A1265, A1277, A1283, A1308 and A1334 (FSANZ 2020, FSANZ 2021, FSANZ 2022a, FSANZ 2022b, FSANZ 2023a, FSANZ 2023b, FSANZ 2024, FSANZ 2025a, FSANZ 2025b).

In these assessments, FSANZ reviewed evidence from 21 clinical trials[1] and cohort studies that measured the effects of 2′-FL either alone or in combination with other HiMO or oligosaccharides on infant growth. Based on the findings from these studies FSANZ concluded that the addition of 2′-FL to infant formula products at concentrations comparable to those normally found in human milk is unlikely to adversely affect normal infant growth.

3.4.3 Current assessment

The applicant provided 7 human studies to support their application. All studies were excluded from the assessment for the following reasons:

- 4 studies did not investigate the effect of the addition of 2′-FL to infant formula on infant growth (Boulangé et al. 2023; Hill and Buck 2023; Giorgetti et al. 2023; Scheuchzer et al. 2023).
- One study did not include a control group (Ramirez-Farias et al. 2024).
- 2 studies were previously assessed by FSANZ in Application A1308 (Jochum et al. 2023) and A1334 (Lazarini et al. 2025).

FSANZ conducted a literature search in PubMed on 01 October 2025[2] to identify any additional studies published since the previous assessment. The search returned 11 studies. Ten of these studies were not considered relevant to include in the nutrition assessment as they did not report the effect of adding 2′-FL to infant formula products on infant growth. One clinical trial was considered.

Yang et al. (2025) investigated the effect of infant formula supplemented with 2′-FL on infantile colic, atopic dermatitis and growth. The prospective, open-label clinical trial studied 338 full-term healthy infants of which 113 infants were breastfed (BM), 111 fed control formula (FF), and 114 fed formula supplemented with 2′-FL (FF_2′-FL) for 1 year. Infants were followed up at birth and at weeks 4, 8, 16, 24, and 52 after delivery. Data collected included body weight, height and head circumference.

Infant anthropometric measures were recorded at baseline and 2 month intervals until 12 months. No significant difference was observed for any measurement including mean body weight at 12 months: 9.3±1.3 (±SD), 9.6±0.93, and 9.5±1.11 kg; mean height: 73.2±1.5, 76.0±2.4, and 75.3±4.9 cm, and head circumference: 44.5±1.1, 45.7±1.4, and 45.8±1.3 cm in the BM, FF, and FF_2′-FL groups respectively ($p > 0.05$). However, several limitations of the study were noted including:

- Lack of randomisation and blinding of participants
- No data relating to loss to follow-up was included
- Exclusive breastfeeding was undertaken for 4 months and full or partial consumption of infant formula was undertaken for 6 months however limited data without statistical analysis was provided at the 4 or 6 month timepoints when complementary feeding may have commenced
- No age at commencement provided.

Due to these limitations the study was not considered in the body of evidence.

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1 (Marriage et al. 2015; Kajzer et al. 2016; Puccio et al. 2017; Sprenger et al. 2017; Reverri et al. 2018; Larsson et al. 2019; Storm et al. 2019; Berger et al. 2020; Lagström et al. 2020; Leung et al. 2020; Román et al. 2020; Vandenplas et al. 2020; Parschat et al. 2021; Ramirez-Farias et al. 2021; Alliet et al. 2022; Cohen 2022; Gold et al. 2022; Lasekan et al. 2022; Vandenplas et al. 2022; Wallingford et al. 2022; Lazarini et al. 2024)

2 Search terms: "2'FL or 2′-FL or 2'-fucosyllactose or 2'fucosyllactose" and "milk or breast or formula" and "anthropometric or weight or growth or development" and "child or infant or baby or maternal" Filters: from 2025/7/23 - 3000/12/12OFFICIAL

3.4.4 Summary of nutrition assessment

FSANZ has previously conducted 9 nutrition assessments on the effect of the addition of 2′-FL to infant formula products on the growth of infants. These assessments found that the addition of 2′-FL to infant formula products at concentrations normally found in human milk does not pose a risk to normal growth of infants.

Since the completion of the previous assessment, one new relevant study was identified that did not find any significant difference in infant growth between infants that consumed formula containing 2′-FL, control formula or were breastfed for up to one year (Yang et al. 2025). However, several limitations in the study design and reporting were identified and consequently the study was not considered in the body of evidence. Therefore, FSANZ maintains the conclusion that 2′-FL added to infant formula products at concentrations found in human milk is unlikely to affect infant growth.

4 Conclusions

FSANZ has previously determined there are no safety concerns associated with the addition of 2′-FL to infant formula products at concentrations up to 2.4 g/L. Newly available information did not indicate a reason to change this conclusion.

Toxicity studies previously reviewed by FSANZ demonstrated 2′-FL is not genotoxic and does not produce adverse effects in short-term oral toxicity studies, including studies using neonatal animal models. In human clinical studies, infant formula products containing 2′-FL was safe and well tolerated. Newly available toxicity studies of the applicant's 2′-FL were consistent with the previously reviewed data. In a 90-day oral toxicity study in rats, the NOAEL was 10.0% (equivalent to 8000 mg/kg bw/day), the highest concentration tested. In a prenatal developmental toxicity study in rats, the NOAEL for maternal and fetal toxicity was 8000 mg/kg bw/day, the highest dose tested. 2′-FL was not genotoxic in vitro or in vivo.

The microbiological assessment undertaken by FSANZ did not identify any public health and safety concerns associated with using GM *E. coli* BL21 as a production organism for 2′-FL. Characterisation of the GM production strain confirmed that all introduced genes were genetically stable and functional.

The associated health benefits from the addition of 2′-FL to infant formula products for infants remain the same: (1) an anti-pathogenic effect; (2) immunomodulation; and (3) development of the gut microbiome through supporting growth of *Bifidobacteria* spp.

FSANZ has previously conducted 9 assessments, consisting of 21 studies, on the effect of the addition of 2′-FL to infant formula products on the growth of infants. These assessments found that the addition of 2′-FL to infant formula products at concentrations normally found in human milk does not pose a risk to normal growth of infants. In the current assessment no new relevant studies were included in the body of evidence. Therefore, FSANZ maintains the conclusion that the addition of 2′-FL to infant formula products at levels typically found in human milk does not pose a risk to normal infant growth.

Based on the available data, there are no public health and safety concerns associated with the addition of 2′-FL from the new source organism to infant formula products at the maximum permitted amount in the Code.

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