FOOD STANDARDS
Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

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Supporting document 1

Risk and technical assessment – Application A1334

A1334 - 2′-FL from GM *Corynebacterium glutamicum* (gene donor: *Corynebacterium urealyticum)* in infant formula products

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Executive summary

Food Standards Australia New Zealand (FSANZ) has assessed an application from Cataya Bio (Shanghai) Co., Ltd to amend the Australia New Zealand Food Standards Code (the Code) to permit a new microbiological source organism for the production of 2′-fucosyllactose (2′-FL). The applicant's 2′-FL is produced by microbial fermentation using a strain of *Corynebacterium glutamicum* containing the gene for alpha-1,2-fucosyltransferase from *Corynebacterium urealyticum*.

Schedule 26 of the Code already permits 2′-FL from several source organisms to be used as a nutritive substance in infant formula products. The maximum permitted 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 was to assess the safety of 2′-FL produced by this new production organism.

The applicant's 2′-FL is chemically, structurally and functionally identical to the naturally occurring substance present in human milk. It is chemically, structurally and functionally identical to 2′-FL previously assessed and permitted by FSANZ. FSANZ's assessment indicates the substance meets the currently approved specification for 2′-FL from *Corynebacterium glutamicum*. The substance is stable under ambient storage conditions.

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

As the applicant's 2′-FL is identical to naturally occurring 2′-FL it is not anticipated there will be any significant differences in pharmacokinetics or safety between naturally occurring and manufactured forms of these substances. Intestinal absorption of HMOs is limited and a significant proportion reaches the large intestine where they are fermented by the microbiota or excreted unchanged in the faeces.

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 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. 2′-FL did not cause acute toxicity and was not genotoxic.

In a 90-day oral toxicity study in rats, the NOAEL was 10% in the diet (equal to 7460 mg/kg bw/day), the highest concentration tested. In a prenatal developmental toxicity study in rats, the NOAEL for maternal and fetal toxicity was 10000 mg/kg bw/day, the highest dose tested.

Previous dietary intake assessments performed by FSANZ have shown that estimated mean and 90th percentile dietary intakes of 2′-FL from infant formula products at the maximum permitted amount in the Code fall within the range of estimated dietary intakes from mature human milk.

FSANZ previously reviewed 20 clinical trials and cohort studies that measured the effect of infant formula containing 2′-FL on infant growth. It was 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 reviewed for the current assessment reported no significant differences in growth between infants consuming infant formula products with or without 2′-FL or breastfed infants. Therefore, FSANZ maintains its previous conclusion.

Based on previous microbiological assessments, given the identical chemical structure and noting the applicant has not requested any change in the maximum permitted amount of 2′-FL added to infant formula products, the associated health benefits from the use of 2′-FL as a nutritive substance in 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.*

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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1 Introduction

Food Standards Australia New Zealand (FSANZ) received an application from Cataya Bio (Shanghai) Co., Ltd to amend the Australia New Zealand Food Standards Code (the Code) to permit a new microbiological source organism for the production of 2′-fucosyllactose (2′-FL). The applicant's 2′-FL is produced by microbial fermentation using a strain of *Corynebacterium glutamicum* containing the gene for alpha-1,2-fucosyltransferase from *Corynebacterium urealyticum*.

Schedule 26 of the Code permits the use of 2′-FL from several microbiological sources. However, it does not include this production strain, which means assessment is required.

2 Food technology assessment

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

FSANZ has assessed applications requesting permissions for Human identical Milk Oligosaccharides (HiMO) in food The information in this section has been built on the assessment of those applications.[1]

2.1 Chemical and physical properties

2′-FL is a human milk oligosaccharide (HMO) found in human milk, composed 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 appears as a white to off-white homogeneous powder that dissolves easily in aqueous solutions but has limited solubility in organic solvents.

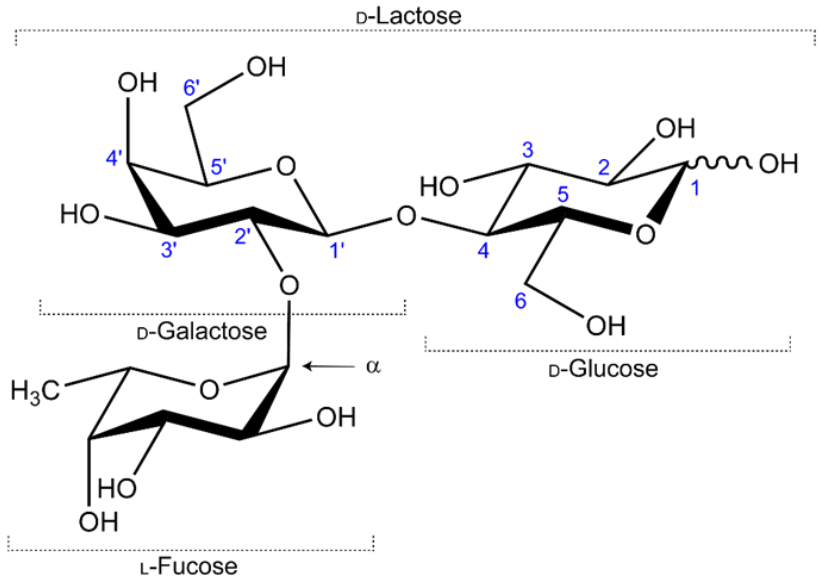

Figure 1 Molecular structure of 2'-FL

[1] The use of 2′-FL in infant formula products has been assessed in applications 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).

2.1.1 Equivalence to human milk 2′-FL

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 method used to determine this was nuclear magnetic resonance (NMR) spectroscopy. In addition, the applicant's 2′-FL was compared with a reference standard using liquid chromatography with mass spectrometry LC-MS/MS) to confirm its identity.

The NMR and LC-MS results confirm that the applicant's 2′-FL is chemically and structurally identical to that occurring naturally in human milk and meets the existing specification in Schedule 3. 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
IUBMB* Chemical name	α-L-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→4)-D-glucopyranose
Alternative common names	2′-O-fucosyllactose 2′-O-L-fucosyl-D-lactose 2′-fucosyl-D-lactose 2′-FL
Alternative names	fucosyl-α-1,2-galactosyl-β-1,4-glucose α-L-Fuc-(1→2)-β-D-Gal-(1→4)-D-Glc
IUPAC abbreviation**	Fuc-α-(1→2)-Gal-β-(1→4)-Glc
CAS registry number***	41263-94-9
Chemical formula	$C_{18}H_{32}O_{15}$
Molecular weight	488.44 g/mol

* The International Union of Biochemistry and Molecular Biology
** The International Union of Pure and Applied Chemistry
*** Chemical Abstract Service

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

Analytical stability data for 5 batches of the applicant's 2′-FL showed stability for at least 12 months under ambient conditions (25°C, 60% RH) and after 26 weeks at accelerated conditions (40°C, 75% RH), equivalent to 2 years.

2.2 Manufacturing processes

The applicant's 2′-FL is produced by microbial fermentation of a GM strain of *C. glutamicum* detailed in section 3.1. The production process consists of 2 stages: upstream processing (USP) and downstream processing (DSP). USP includes fermentation in a medium

containing a yeast extract, soy peptone, trace elements and controlled parameters including temperature, pH, dissolved oxygen and aeration.

DSP includes purification by removing impurities sequentially, followed by drying. The large molecules are removed by ultrafiltration followed by further concentration. Active charcoal is used to removed colour and organic matter with electrodialysis and ion exchange to remove inorganic salts. Saccharide impurities are removed by chromatography followed by spray drying to produce 2′-FL.

According to the applicant, their production process complies with current Good Manufacturing Practice and Hazard Analysis of Critical Control Points plan. Their manufacturing process and food safety management systems also meet relevant food safety systems certification, the International Organisation for Standardisation 9001 and GMP under US FDA and the Food Safety Modernisation Act 2011.

2.3 Specifications

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 *C. glutamicum* in S3—51 from a different production organism.

The applicant provided a proposed specification for its 2′-FL that aligns with the existing specification for 2′-FL from *C. glutamicum* in S3—51. (see Table 3). The specification includes parameters for the identity of 2-FL and impurities.

The S3—51 specification aligns with the specification for 2′- FL from *C. glutamicum* for the European Union (EU), the United Kingdom (UK) and Scotland as these are based on the same European Food Safety Authority (EFSA) assessment.

The applicant submitted analytical data for 5 separate batches of 2′-FL which showed that the applicant's 2′-FL meets the currently approved specification for 2′ -FL in S3—51 (see Table 3). Ethanol was excluded from the analysis because it is neither used by the applicant nor produced by the organisms, and therefore not required.

2.3.1 Impurities

Section 1.1.1—15 of the Code states that any substance employed as a nutritive ingredient must comply with the applicable identity and purity specifications outlined in Schedule 3. Schedule 3 includes a specification for 2′-FL sourced from *C. glutamicum*.

The analytical results for 5 independent representative batches of the applicants 2′-FL provided in the application indicate that it meets the requirements in the comparable specification for 2′-FL sourced from *C. glutamicum* in S3—51.

The applicant's product contains a minimum 94% w/w of 2′-FL. There are smaller quantities of other carbohydrates present, including D-lactose, 3-Fucosyllactose, difucosyl-D-lactose, D glucose and D-galactose. The applicant's analytical results meet the comparable specification for 2′-FL in S3—51.

The production microorganism is removed during 2′-FL processing and purification. Polymerase chain reaction (PCR) tests confirmed no residual DNA from the microorganism is present in the final product.

Table 2: The specification for 2'-FL from* Corynebacterium glutamicum *(as currently approved in S3—51)

Parameter	**Specification**
Chemical name	α-L-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→4)-D-glucopyranose
Chemical formula	$C_{18}H_{32}O_{15}$
Molecular weight	488.44 g/mol
CAS number	41263-94-9
Description	White to off-white ivory powder
2′-FL	not less than 94.0% (water free)
D-lactose	not more than 3.0% (water free)
L-fucose	not more than 3.0% (water free)
3-fucosyllactulose	not more than 3.0% (water free)
difucosyl-D-lactose	not more than 2.0% (water free)
glucose	not more than 3.0% (water free)
galactose	not more than 3.0% (water free)
water	not more than 9.0%
ash, sulphated	not more than 0.5%
ethanol	not more than 1,000 mg/kg (for crystallised product from solvent only)
residual proteins	not more than 0.005%
lead	not more than 0.02 mg/kg
arsenic	not more than 0.03 mg/kg
cadmium	not more than 0.01 mg/kg
mercury	not more than 0.05 mg/kg
Microbiological	
total plate count	not more than 500 cfu/g
coliforms	not more than 10 cfu/g
yeasts and moulds	not more than 100 cfu/g
aflatoxin M1	not more than 0.025 ug/kg
residual endotoxins	not more than 10 EU/mg

2.4 Analytical methods for detection

The applicant has developed an internal and ISO validated method for detecting and quantifying 2′-FL using high performance liquid chromatography coupled with refractive index detector (HPLC-RID).

2.5 Food technology conclusion

From assessment of the data provided in the application, the applicant's 2′-FL produced by a microbial fermentation manufacturing process is chemically and structurally identical to the 2′-FL naturally occurring in human milk and to 2′-FL previously assessed and permitted by FSANZ.

The results from analytical testing demonstrate the applicant's product meets the currently approved specification for 2′-FL from *C. glutamicum*. This specification aligns with current specifications for the applicant's 2′-FL in EU and UK legislation.

The results at 12-months for the ambient shelf-life trial, and at 26-months for the accelerated study showed that the applicant's 2′-FL is stable under ambient storage conditions.

3 Safety assessment

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

3.1 GM production strain assessment

3.1.1 Host organism

C. glutamicum is a non-pathogenic, gram-positive, rod-shaped soil bacteria originally isolated in the late 1950s (Joo-Young et al. 2016, Hirasawa et al. 2018, Li et al. 2025). The organism has a biotechnological history spanning over 60 years, with industrial scale commercialisation first recorded in the 1960s (Sgobba et al. 2018, Park and Lim 2024).

Since then, *C. glutamicum* has been used extensively for the production of amino acids, and a diverse range of other biomolecules (e.g. diamines, organic acids, carotenoids, proteins and biopolymers). These have been achieved through both metabolic pathway restrictions and genetic modification (Sgobba et al. 2018, Tsuge and Yamaguchi 2021). Due to its industrial importance and versatility, *C. glutamicum* has been intensively studied and documented (Joo-Young et al. 2016). As a result of this extensive study, EFSA has granted *C. glutamicum* Qualified Presumption of Safety (QPS) status for production purposes (EFSA BIOHAZ Panel 2025).

Whole genome sequencing (WGS) data provided by the applicant confirmed the identity of the production organism as *C. glutamicum*. The production organism has been genetically modified by inserting the gene for alpha-1,2-fucosyltransferase from *Corynebacterium urealyticum* into the *C. glutamicum* wild type strain (ATCC 13032). See section 3.1.2 (Characterisation of the GM production organism) for more detail.

Analysis of 3 representative batches of the final preparation, along with described methodology, demonstrated neither viable cells nor DNA of the production organism were detected in the final product.

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

3.1.2 Characterisation of the GM production organism

3.1.2.1 Development of the GM production strain

The *C. glutamicum* production strain was generated by modifying the wild type *C. glutamicum* strain ATCC 13032 with several genes that direct the biosynthesis of 2′-FL. The genes were all synthesised *de novo*, ensuring that no genetic material other than the desired genes and non-coding genetic elements were introduced into the production strain.

A total of 5 heterologous genes were introduced into the genome of the host strain by homologous recombination. These encode and were sourced from:

- GDP-D-mannose-4,6-dehydratase from *E. coli*;
- GDP-4-keto-6-deoxymannose reductase from *Campylobacter jejuni*;
- GDP-D-rhamnose epimerase from *Oryza sativa*;
- Lactose permease from *E. coli*; and
- α-1,2-fucosyltransferase from *C. urealyticum*.

Together, these genes facilitate the production of GDP-fucose, the uptake of lactose into the cell, and the conversion of lactose and GDP-fucose into 2′-FL. The roles of the introduced genes in the biosynthesis of 2′-FL are shown in Figure 2.

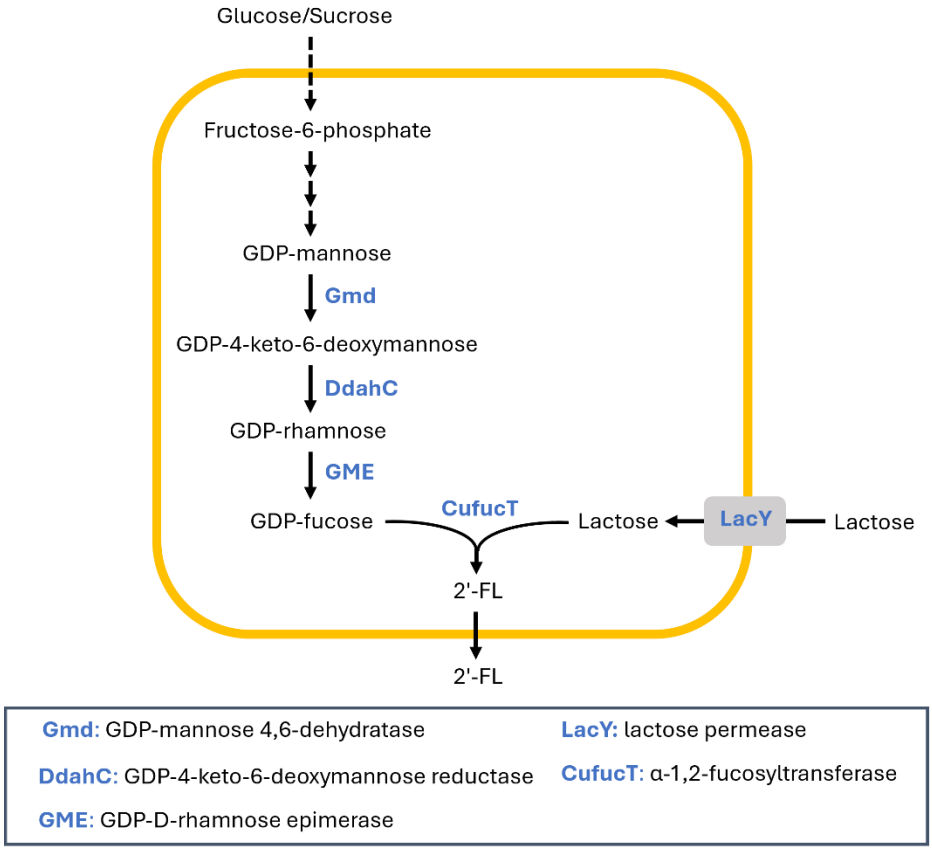

Figure 2. *The role of the 5 introduced genes in directing the biosynthesis of 2′-FL in the production strain. LacY facilitates transport of lactose into the cell; Gmd, DdahC and GME facilitate the conversion of GDP-mannose into GDP-fucose; and CufucT catalyses the conversion of lactose and GDP-fucose into 2′-FL.*

3.1.2.2 Characterisation of introduced DNA

PCR, Sanger sequencing, and WGS were 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. WGS also confirmed the absence of antibiotic resistance markers in the final production strain.

3.1.2.3 Genetic stability and inheritance of the introduced DNA

Data was provided on the growth rate and 2′-FL production over 5 consecutive subculturing passages. These data showed that the growth rate and 2′-FL yield over the 5 passages was stable, indirectly confirming the stability and inheritance of the inserted DNA over multiple generations.

3.1.3 Conclusion

FSANZ's microbiological risk assessment did not identify any public health and safety concerns associated with the use of *C. glutamicum* 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 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.

These assessments found 2′-FL to be 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 containing 2′-FL was safe and well tolerated.

3.2.2 Newly available data

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

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

All tests were conducted in China Metrology Accreditation (CMA) accredited laboratories, at the Hunan Institute for Occupational Disease Prevention Institute, 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 (SAC), Beijing).

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 (generally consistent with OECD Test Guideline (TG) 401 (1987))

In an acute oral toxicity study, 10 male and 10 female SD rats were administered a single dose of 10,000 mg/kg bw 2′-FL and monitored for 14 days. All animals survived to the end of the study and no clinical signs of toxicity were observed. No abnormalities were found in gross pathology examinations at the end of the 14-day observation period. The LD_{50} of 2′-FL was > 10,000 mg/kg bw in this study.

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 (generally consistent with OECD TG 408 (2018))

In a 90-day oral toxicity study, SD rats (10/sex/group) were administered 2′-FL in the diet at concentrations of 0, 2.5%, 5% and 10% (equal to 0, 2280, 4510 and 9160 mg/kg bw/day in males and 0, 1890, 3670 and 7430 mg/kg bw/day in females, respectively). In addition, mid-term satellite control and high-dose groups (5/sex/group) were fed the test item for 45 days, and recovery control and high-dose groups (5/sex/group) were fed control feed for 28 days following the 90-day treatment period. Mortality, clinical signs, body weight and body weight gain, food consumption and food efficiency were recorded throughout the study. Ophthalmic examination was conducted before and at the end of the 90-day test period. Haematology, clinical chemistry and urinalyses examinations were performed at 45 days (mid-term satellite groups), 90 days and at the end of the recovery period. All animals in the main study and recovery groups underwent gross and histopathological examination at termination.

All animals survived to the end of the treatment or recovery period. There were no treatment-related effects on any of the test parameters. The no observed adverse effect level (NOAEL) in this study was 10% in the diet (equal to 9160 mg/kg bw/day in males or 7460 mg/kg bw/day in females), the highest concentration tested.

Teratogenicity test in rats (CCI) Regulatory status: conducted in a CMA-accredited laboratory, in accordance with National Food Safety Standard GB 15193.14-2015 (generally consistent with OECD TG 414 (2018)).

In a prenatal developmental toxicity study, 85 male and 85 nulliparous female (11–12 weeks old) SD rats were paired at a 1:1 ratio for mating, following 5 days of acclimation. Female rats were observed daily for successful mating. Mated females were classified as day 0 of gestation on the day of confirmed mating. From day 6 to 15 of gestation, mated females (20/group) were administered 2′-FL in purified water by via oral gavage at doses of 0, 2500, 5000 or 10000 mg/kg bw/day. Daily observations of indicators of miscarriage and premature birth (including clinical observations of skin, fur and eyes) were recorded throughout the study. Mortality, body weight and weight gain, food consumption, and activity were further recorded throughout the study. All animals underwent necroscopy for the assessment of maternal and fetal toxicity at termination (day 20 of gestation).

Pregnancy endpoints assessed at termination included the number of maternal animals, corpora lutea, implantations and live fetuses, the live fetus, stillborn fetus and resorbed fetus rates, and the sex ratio in each treatment group. Uterine weight (including fetus/es), placental weight, and the total and net weight gain of maternal animals were assessed. The study did not include the measurement of thyroid hormone (TSH, T4 and T3) levels in maternal animals, deviating from the current version of OECD TG 414.

Fetal growth and development endpoints assessed at termination included body weight, body length, and tail length of fetal rats in each treatment group. External, internal organ, and skeletal malformations in fetal rats were examined and the total malformation rate of fetuses per litter was determined for each treatment group. The study did not include the measurement of anogenital distance, another deviation from OECD TG 414.

All maternal animals survived to the end of the treatment period. There were no treatment-related clinical signs or adverse effects on any of the maternal parameters evaluated.

There were no treatment-related adverse effects on any of the parameters evaluated, indicating no teratogenic effect of the treatment on fetal rats. The NOAEL for maternal toxicity and for fetal toxicity was 10000 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 CMA accredited laboratories in accordance with Chinese testing requirements. The studies 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.

The results of these studies, as summarised in Table 3, showed no evidence of mutagenicity, clastogenicity or aneugenicity.

Table 3: Genotoxicity studies of 2'-FL

Test[1]	**Test object**	**Concentration**	**Results**
Bacterial reverse mutation test (GB 159193.4-2014; OECD TG 471 [2020])	Salmonella typhimurium strains TA97a, TA98, TA100, TA102 & TA1535	0, 50, 158, 500, 1582 or 5000 µg/plate (initial test) 0, 8, 40, 200, 1000 or 5000 µg/plate (confirmation test)	Negative ± S9[2]
In vitro mammalian chromosomal aberration test (GB 15913.23-2014; OECD TG 473)	Chinese hamster lung (CHL) cells	0, 1250, 2500 or 5000 µg/mL	Negative ± S9[3]
In vivo mammalian erythrocyte micronucleus test (GB 15193.5-	Kunming mice bone marrow	0, 2500, 5000 or 10,000 mg/kg bw	Negative[4]

2014; OECD TG 474)			

[1] Study references are CCI.
[2] Tests performed in triplicate following plate incorporation method.
[3] Study deviated from OECD TG 473 as only 100 metaphases were scored per treatment rather than 300 as recommended. However, positive controls produced the expected response.
[4] Study had several deviations from OECD TG 474: Bone marrow was collected 6 hours after the last treatment rather than the recommended 18 – 24 hours. 2000 polychromatic erythrocytes per animal were scored for micronuclei, and only 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.

3.2.3 Safety assessments by other agencies

As noted in previous FSANZ assessments, 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's 2′-FL has been submitted to the European Union (EU) for the Amendment of the Union List of Novel Food Specifications for 2′-FL (microbial source). The status of this application (novel food application no. EFSA-Q-2025-00169) is currently listed by EFSA as under ongoing risk assessment.

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 that this 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 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 for infants 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 addition of 2′-FL to infant formula products as a nutritive substance is currently permitted up to 96 mg/100 kJ, equivalent to 2.4 g/L. The current application is to permit a new source organism for the production of 2′-FL, with no other change requested.

3.4.2 Previous FSANZ assessments of 2′-FL

FSANZ has previously assessed the effect of 2′-FL in infant formula products on infant growth in 8 applications: 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).

For these assessments FSANZ considered the evidence from 20 clinical trials and cohort studies that measured the effect of 2′-FL alone and in combination with other HiMOs or oligosaccharides on infant growth[2]. From these studies it was concluded that 2′-FL added to infant formula products is unlikely to affect normal growth of infants when added at levels that are found in human milk.

3.4.3 Current assessment

The applicant did not provide any evidence for the effect of 2′-FL added to infant formula products on infant growth. FSANZ undertook a Pubmed search on 23 July 2025 to identify any new studies since the previous 2′-FL assessment was undertaken[3]. Thirty-nine studies were identified, of which one clinical tri.al and one systematic review were considered. The systematic review was screened for any relevant studies that had not previously been considered by FSANZ, however none were identified (Hojsak et al. 2025). One clinical trial was included in the body of evidence (Lazarini et al. 2024).

Lazarini et al. (2024) measured the effect of infant formula products containing 2′-FL (1 g/L), galacto-oligosaccharides (GOS) and fructo-oligosaccharides (FOS) compared to formula containing only GOS and FOS (4 g/L in 9:1 ratio), in otherwise nutritionally identical bovine milk-based formula, or a breastfed control group on infant growth, in a randomised, double-blind, controlled trial. Healthy full-term infants were randomly divided into 2 groups (n=30 each) at enrolment (less than 1 month of age) and consumed the allocated formula for 3 months. A breastfed reference group (n=30) was also included.

Anthropometric measurements including weight, length, and head circumference were recorded at baseline and at 3 monthly visits. Mean weight gain (final weight – weight at admission) between baseline and 4 months of age was the primary outcome, and anthropometric z-scores as secondary outcomes. One-way ANOVA was used to determine statistically significant difference between the 3 groups.

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[2] 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 and Wallingford et al. 2022.

[3] 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 2024/8/24 - 3000/12/12

Mean weight gain was not significantly different between groups, 2937 ± 363 g, 2760 ± 353 g and 2810 ± 570 g for the 2′-FL, control formula and breastfed group respectively; p = 0.287. Similarly, length gain was not significantly different between groups, 11.0 ± 1.5 cm, 10.7 ± 1.7 cm, 10.9 ± 1.8 cm for the 2′-FL, control formula and breastfed group respectively; p = 0.745. Weight-for-age, length-for-age, head circumference-for-age, and BMI-for-age z-scores for the groups were no more than ± 0.3 and therefore within the normal range defined as up to 2 standard deviations below and up to one standard deviation above the median (WHO 2008). The weight-for-age z-score in the 2 infant formula groups was not significantly different (0.0 ± 0.39 and -0.2 ± 0.56; p = 0.09) although it reached significance across the 3 groups (p = 0.041). Length-for-age, BMI-for-age, and head circumference-for-age z-scores were not significantly different between the 3 groups (p > 0.05).

3.4.4 Summary of nutrition assessment

FSANZ has assessed the effect of infant formula products containing 2′-FL at concentrations up to 2.4 g/L on infant growth in 8 previous applications. It was concluded that 2′-FL added to infant formula products at concentrations normally found in human milk did not affect infant growth. In the present application FSANZ identified one additional study that measured the effect of infant formula containing 1 g/L 2′-FL on infant growth. No significant difference was observed in the majority of growth endpoints, including mean weight and length gain, length-for-age, BMI-for-age, head circumference-for-age z-scores. Although a significant difference was observed for weight-for-age z-score across the 3 groups, no difference was observed between the 2 infant formula groups. All z-score measurements were within normal growth range as defined by the WHO. 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

Schedule 26 of the Code currently permits the use of 2′-FL from different source organisms as nutritive substances in infant formula products. The maximum permitted amount is 96 mg/100 kJ, equivalent to 2.4 g/L. The purpose of the present assessment was therefore to assess the safety of 2′-FL produced by the new production organism.

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.

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

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.

As the applicant's 2′-FL is identical to naturally occurring 2′-FL it is not anticipated there will be any significant differences in pharmacokinetics or safety between naturally occurring and manufactured forms of these substances.

Intestinal absorption of HMOs is limited and a significant proportion reaches the large intestine where they are fermented by the microbiota or excreted unchanged in the faeces.

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 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. 2′-FL did not cause acute toxicity and was not genotoxic. In a 90-day oral toxicity study in rats, the NOAEL was 10% in the diet (equal to 7460 mg/kg bw/day), the highest concentration tested. In a prenatal developmental toxicity study in rats, the NOAEL for maternal and fetal toxicity was 10000 mg/kg bw/day, the highest dose tested.

Previous dietary intake assessments performed by FSANZ have shown that estimated mean and 90th percentile dietary intakes of 2′-FL from infant formula products at the maximum permitted amount in the Code fall within the range of estimated dietary intakes from mature human milk.

FSANZ considered the effect of 2′-FL in infant formula on infant growth. One new study identified since the previous assessment did not change the conclusion that 2′-FL added to infant formula products at concentrations found in human milk is unlikely to pose a risk to infant growth.

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.

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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