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Supporting document 1

Risk and technical assessment – Application A1251

A1251 – 2'-FL combined with galacto-oligosaccharides and/or inulin-type fructans in infant formula products

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Executive summary

FSANZ has assessed an application from Nutricia Australia Pty Ltd (Nutricia) and Chr. Hansen A/S to amend the Australia New Zealand Food Standards Code (the Code) to permit the addition of 2′-fucosyllactose (2′-FL) in combination with galacto-oligosaccharides (GOS) and/or inulin-type fructans (ITF) in infant formula products (IFP). ITF includes substances such as fructo-oligosaccharides (FOS), short-chain FOS (scFOS), long-chain FOS (lcFOS), oligofructose and inulin.

While 2′-FL, GOS and ITF are all permitted to be added to infant formula products, section 2.9.1—7(2) of the Code currently prohibits the addition of 2′-FL to infant formula products containing GOS or ITF. No changes are requested to individual permissions for 2'-FL, GOS and ITF in infant formula products, which include maximum permitted use levels.

The safety, technological, nutritional impact and beneficial health effects from individual addition of these ingredients to infant formula products have previously been considered (Applications A1155, A1190 and A1233 for 2′-FL; Proposal P306 for GOS/ITF and A1055 for scFOS). The purpose of this assessment is to consider the combination of these ingredients. Previous assessment found that 2′-FL is stable, structurally and chemically identical to naturally occurring 2′-FL and free from fermentation derived contaminants (FSANZ 2019). Information has been provided to assess the stability of the blended ingredients with FSANZ confirming that the ingredients provide adequate shelf-life and stability.

FSANZ has previously determined that there are no safety concerns associated with the addition of 2′-FL, GOS and/or ITF to infant formula products at concentrations up to 2.4 g/L for 2′-FL, 3 g/L for ITF and 8 g/L for GOS alone or in combination with ITF (up to a maximum of 3 g/L ITF). These conclusions were supported by toxicological studies in laboratory animals and clinical studies in infants which found no adverse effects from the use of these substances.

FSANZ has previously concluded that 2′-FL added to IFP should not affect growth at levels normally found in human milk. In addition, FSANZ has previously assessed the addition of a total level of 8 g/L of GOS and ITF, alone or combined at any ratio, in IFP and concluded that a maximum of 8 g/L in IFP is unlikely to pose a risk to the growth and development of infants from birth onwards.

A newly available clinical study reviewed by FSANZ for this assessment found that consumption of infant formula containing 2′-FL (1 g/L) in combination with a 9:1 ratio of short-chain GOS (scGOS) and lcFOS (8 g/L) was safe, well tolerated and did not affect growth, although some limitations in study design in terms of assessment of growth were noted.

Taken together, the available evidence supports the conclusion that no difference in growth is likely to occur in infants fed IFP that contains 2′-FL, GOS and/or ITF at previously permitted levels.

The limited evidence available from human intervention studies raised no potential microbiological safety concerns from a combination of 2'-FL with GOS and/or ITF in IFP at the levels proposed by the Applicant.

Dietary intakes of 2′-FL in combination with GOS and/or ITF from IFP were estimated for infants using a model diet approach. Assuming the addition of 2′-FL with GOS and/or ITF at the maximum permitted levels in the Code (96 mg/100 kJ and 290 mg/100 kJ respectively), the estimated mean and 90th percentile (P90) dietary intakes of 2′-FL combined with GOS and/or ITF from infant and follow-on formula ranged between 5 and 17 g/day. These intakes were lower than the estimated mean and P90 intakes of human milk oligosaccharides from human milk.

Given the absence of any identifiable hazard in toxicological and clinical studies with 2′-FL, GOS and/or ITF, alone or in combination, and noting that estimated exposures are lower than those of human milk oligosaccharides from human milk, there are no safety concerns from the addition of 2′-FL in combination with GOS and/or ITF to IFP at the proposed levels.

No human intervention studies investigating a bifidogenic or anti-pathogenic health effect of the combination of 2′-FL with GOS and/or ITF were provided by the Applicant or identified by FSANZ. Results from *in vitro* and animal studies of combinations of 2′-FL and GOS and/or ITF are consistent with beneficial health effects observed for the individual components and provide some indication of mechanisms involved. However, they do not allow any conclusions to be drawn on whether there are any additional benefits arising from supplementation with a combination of 2′-FL and GOS and/or ITF.

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1 Introduction

FSANZ received an application from Nutricia Australia Pty Ltd (Nutricia) and Chr. Hansen A/S to amend the Australia New Zealand Food Standards Code (the Code) to permit the addition of 2′-fucosyllactose (2′-FL) in combination with galacto-oligosaccharides (GOS) and/or inulin-type fructans (ITF[1]) in infant formula products (IFP). ITF includes substances such as fructo-oligosaccharides (FOS), short-chain FOS (scFOS), long-chain FOS (lcFOS), oligofructose and inulin.

While 2′-FL, GOS and ITF are all permitted to be added to IFP, section 2.9.1—7(2) of the Code currently prohibits the addition of 2′-FL to IFP containing GOS and/or ITF.

The Applicants intend to combine a scGOS/lcFOS mixture (ratio of 9:1; levels up to 8 g/L) with Chr. Hansen's 2′-FL (levels up to 2.4 g/L) to Nutricia IFP. No changes are requested to individual permissions for 2′-FL, GOS and ITF in IFP, which include maximum permitted use levels.

The safety, technological, nutritional impact and beneficial health effects from individual addition of these ingredients to infant formula products have previously been considered (Applications A1155, A1190 and A1233 for 2′-FL produced by a range of genetically modified (GM) production strains; Proposal P306 for GOS/ITF and A1055 for scFOS[2]). The purpose of the present assessment is to consider the combination of these ingredients.

2 Food technology assessment

The food technology assessment provides technical information on the chemical identification, physicochemical properties and specifications for the combination of 2′-FL and the GOS/ITF mixture when added to IFP. 2′-FL, GOS and ITF have previously been assessed in terms of their food technology functions, with FSANZ having no concerns.

2.1 Chemical properties

2.1.1 Chemical names, properties, and structures

The chemical and physical properties of the individual 2′-FL, GOS and ITF ingredients can be found in A1190 and P306, Attachment 4 – Technical and Chemical Assessment (FSANZ 2019, FSANZ 2008a). The ingredients that will be combined in IFP are those approved from A1190 and A1055 which considered the addition of short-chain fructo-oligosaccharides (scFOS) to infant formula, infant foods and supplementary foods for young infants[3] as an alternative to inulin-derived substances (FSANZ 2013a). Combining the ingredients in IFP does not change their properties. IFP manufacturers must ensure final products meet GMP and are safe. ensure final products meet GMP and are safe.

Within A1190 the terms 2′-FL_{micro} and 2′-FL_{chem} are used to refer to two different forms of 2′-FL. 2′-FL_{chem} is produced by chemical synthesis and 2′-FL_{micro} is produced by microbial fermentation (FSANZ 2019). 2′-FL_{micro} is the type referred to within the Application and this assessment.

2.1.2 Structural identification

[1] The term inulin-type fructans (ITF) replaced the term inulin-derived substances (IDS) following Application 1055 (FSANZ 2013b)
[2] See reference list for links to these applications and Proposal P306 (FSANZ 2008a; 2008b; 2013a; 2013b; 2019; 2020; 2021; 2022)
[3] Less than three months as older infants are defined as 4-6 months.

A1190 confirmed that 2′-FL_{micro} is chemically and structurally identical to the naturally occurring substances in human milk and 2′-FL_{chem} (FSANZ 2019). A1055 and P306 confirmed the structural and chemical identification of the GOS and/or ITF mixture (FSANZ 2008a).

2.2 Impurity profile

Both Nutricia Australia Pty Ltd (Nutricia) and Chr. Hansen A/S have established procedures in place to ensure the purity of the 2′-FL, GOS and/or ITF ingredients. These procedures were provided as part of A1190, P306 and A1055 and were approved in both instances (FSANZ 2019, FSANZ 2008a, FSANZ 2013a). Removing the prohibition in the Code to permit the addition of 2′-FL with GOS and/or ITF in IFP does not remove the requirements for ingredients to be produced according to GMP.

2.3 Manufacturing processes

The manufacturing process for 2′-FL is described in Application A1190. For GOS, ITF and/or scGOS are syrups produced by converting lactose into galacto-oligosaccharides, whereas lcFOS is a powder produced by the fractionation of inulin. Both are soluble fibres and supplied to Nutricia by various suppliers.

Nutricia and Chr. Hansen A/S has provided information regarding the manufacturing process for blending the ingredients in IFP, the details of which have been provided to FSANZ as Confidential Commercial Information. However, a non-confidential summary has been provided in the Application, which covers the following main steps:

- A spray dried base powder is produced, using pasteurised bovine milk and other functional ingredients, food additives and processing aids.
- The base powder is blended with the remaining infant formula ingredients.
- Packaging of final product.

All processes are completed in facilities that are registered and approved for dairy manufacturing, HACCAP certified and as such are subject to regular government audits. The facilities are ISO and accredited food safety systems certified.

2.3.1 Stability

Nutricia and Chr. Hansen A/S have provided updated shelf-life studies for IFP containing both 2′-FL and the scGOS/lcFOS mixture (Nutricia Research 2021). The study found that 2′-FL, at levels between 2.9 – 4.0 g/L, combined with 8 g/L scGOS & lcFOS is stable for at least 18 months at 20^{o}C and 30^{o}C in powder form. The shelf-life studies were conducted on IFPs manufactured in Germany and were done on products manufactured to upgrade to new legislation in the EU. Analytical results of the 2'-FL (at both ranges) remained consistent at 20C and 30C for up to 18 months. The Applicant is aware that the Code permits a maximum use level of 2.4 g/L and are not seeking to increase this amount in this Application. Danone Nutricia can confirm that it intends to use up to the maximum level permitted in the Code at 2.4g/L and not the higher levels tested in Europe based products.

The combination of 2′-FL with GOS and/or ITF is already approved for use in several countries within Europe and Asia. Additionally, 2′-FL, GOS and ITF are used individually or in some combination in Europe, USA, South America and Asian countries in IFP manufactured by other companies.

In the United States, the inclusion of 2′-FL and/or GOS in infant formula has received GRAS notification, i.e., the Applicant received 'no questions asked' letters. Shelf-life studies included in the GRAS notice reveal comparable results to the study provided by Nutricia. 2′-FL (GOS already present) was found to be stable for up to 104 weeks when stored at 25 °C and 60% humidity, and for 26 weeks (6 months) when stored at 40 °C and 75% humidity. Shelf-life studies in GRN 546, GRN 650, GRN 735 and GRN 749 produced comparable results (FDA 2020).

2.4 Product specifications

A specification for Chr. Hansen's 2′-FL is included in Schedule 3 of the Code. Nutricia intends to use this 2′-FL ingredient, meaning a new specification is not required. The scGOS/lcFOS mixture at a ratio of 9:1 ratio has been used as an ingredient in IFP in Australia and New Zealand for approximately thirteen years. A specification for GOS and/or ITF is not included in Schedule 3 identity and purity. The existing provisions for Schedule 3 do not require amendment for the intended use of these ingredients, separately permitted, when added to IFP.

Certificates of analysis were provided for 2′-FL with A1190 and GOS and/or ITF with P306 and A1055.

2.5 Analytical method for detection

Analytical methods for detection are available in detail in the previously considered Application and Proposal for both 2′-FL (A1190) and GOS and/or ITF (A1055 and P306).

The methods provided by Nutricia and Chr. Hansen A/S were approved by FSANZ as part of A1190, A1055 and P306.

2.6 Food technology conclusion

The individual use of, 2′-FL or GOS and/or ITF, have been individually approved for use by FSANZ under A1190, A1055 and P306. The food technology assessment of this Application, to allow the combination of 2′-FL with GOS and/or ITF, remains consistent with the findings in A1190, A1055 and P306 (FSANZ 2019, FSANZ 2013a, FSANZ 2008a). The previous Applications and Proposal found that 2′-FL_{micro} (subject of this Application) is stable, structurally and chemically identical to naturally occurring 2′-FL and free from fermentation derived contaminants (FSANZ 2019). Information has been provided to assess the stability of the blended ingredients with FSANZ confirming that the blend of ingredients provides an adequate shelf-life. International studies of shelf-life and stability further confirm the information provided by Nutricia and Chr. Hansen A/S. No other food technology concerns were identified.

3 Safety assessment

3.1 Toxicology assessment

3.1.1 Previous assessments of 2′-FL by FSANZ

FSANZ has previously assessed the safety of 2′-FL produced by Chr. Hansen under A1190 (FSANZ 2021a). The safety assessment concluded that no potential safety concerns were identified in the assessment of the genetically modified *Escherichia coli* BL21 strains used in the production of Chr. Hansen's 2′-FL.

In addition, the safety of 2′-FL produced from other sources has been assessed in Applications A1155 and A1233 (FSANZ 2019; FSANZ 2020; FSANZ 2022).

There is an extensive toxicological database for 2′-FL, and data derived using 2′-FL produced from a range of sources have been evaluated by FSANZ, as well as studies of 2′-FL in combination with other oligosaccharides found in human milk. The 2′-FL from all these sources was demonstrated to be chemically and structurally identical to human 2′-FL.

2′-FL was not genotoxic *in vitro* or *in vivo*. No adverse effects were observed in multiple subchronic oral toxicity studies in neonatal rats at doses up to 5000 mg/kg bw/day, or in juvenile and adult rats at doses > 7000 mg/kg bw/day. Studies in neonatal piglets administered formula containing 2′-FL at concentrations up to 4 g/L also found no adverse effects. In human studies, infant formula supplemented with 2′-FL was well tolerated with no significant increases in adverse events. 2′-FL was also well tolerated in studies with children and adults.

Based on the available data, FSANZ has concluded there are no safety concerns associated with the addition of 2′-FL to infant formula products at concentrations up to 2.4 g/L, which is within the range of naturally occurring levels in human milk from the majority of women (0.6 – 7.8 g/L; FSANZ 2021a).

3.1.2 Previous assessments of GOS and/or ITF by FSANZ

In its evaluation of P306 – *Addition of Inulin/FOS & GOS to food* – FSANZ concluded that the addition of a total level of 8 g/L of inulin-derived substances, hereafter referred to as ITF[4], and/or GOS, alone or in combination at any ratio, to infant formula products is unlikely to pose a risk to young infants (FSANZ 2008a). This conclusion was based on data from clinical trials which provided formulas supplemented with up to 10 g/L of ITF and GOS to infants without evidence of harm. Data also indicated these oligosaccharides are fermented to a similar or greater extent than human milk oligosaccharides (HMOs). The safety of the maximum level was further supported by the presence of higher levels of HMOs, up to 25 g/L, in human milk.

Although no safety concerns were identified for the addition of ITF at concentrations up to 8 g/L, the maximum amount of these substances that could be added to infant formula was set at 3 g/L, noting this level meets the needs of infant formula manufacturers. For GOS, the maximum permitted level was 8 g/L alone or in combination with ITF (up to a maximum of 3 g/L ITF in the combination).

More recently, FSANZ's assessment of A1055 – *Short-chain fructo-oligosaccharides* – concluded that sucrose-derived scFOS, like inulin-derived scFOS, are safe to add to infant formula products up to the maximum level established in the previous permission (FSANZ 2013a). Studies in laboratory animals at doses far in excess of those likely to be encountered by infants and young children found no identifiable hazard.

Several subchronic toxicity studies of 2′-FL or LNnT in neonatal animal models reviewed for A1155 and A1190 also included a reference group treated with FOS at doses of either 5000 or 6000 mg/kg bw/day. No treatment-related adverse effects were observed in these studies.

[4] The term 'inulin-derived substances' in the Code was later changed to 'inulin-type fructans' under application A1055.

3.1.3 2′-FL in combination with GOS and/or ITF

FSANZ's assessment of Application A1155 included several clinical studies with infants fed formula containing various combinations of 2′-FL and scFOS or GOS:

- 0.2 g/L 2′-FL and 2 g/L scFOS (Reverri et al 2018; Kajzer et al 2016)
- 0.2 g/L 2′-FL and 1.8 g/L scFOS (Reverri et al 2018)
- 0.2 g/L 2′-FL and 2.2 g/L GOS; plus 1 g/L 2′-FL and 1.4 g/L GOS (Marriage et al 2015).

In all of these studies the intervention was found to be well tolerated with no safety concerns identified.

3.1.4 Newly available data

The Applicant submitted a publication reporting the results of a clinical study of infant formula containing a combination of 2′-FL and scGOS/lcFOS. This study is reviewed below. The Applicant also identified several other clinical studies of IFP containing 2′-FL in combination with scFOS, GOS or LNnT. These studies were previously reviewed by FSANZ as part of the assessment of A1155.

To further support the Application, the Applicant conducted literature searches for studies on the safety of GOS and FOS or ITF added to IFPs. A range of studies were identified, including 10 involving the 9:1 combination of scGOS/lcFOS proposed for use by the Applicant. As these studies did not evaluate GOS and/or ITF in combination with 2′-FL they are of limited relevance to the present Application and are not reviewed below; however infant formula containing GOS/ITF was reported to be safe and well tolerated in these studies.

Clinical study of partly fermented infant formula containing 2′-FL in combination with scGOS/lcFOS (Vandenplas et al 2020)

In a double-blind, randomised, controlled, multi-country, two-arm parallel group trial, 215 formula-fed term infants aged ≤ 14 days were randomised to receive a test formula (n = 108) containing 26% fermented formula with postbiotics derived from a Lactofidus™ fermentation process (including 3′-galactosyllactose [3′-GL]), 8 g/L scGOS/lcFOS (9:1), 1 g/L 2′-FL, and milk fat), or a control formula (n = 107) containing 8 g/L scGOS/lcFOS until 17 weeks of age. A group of 61 breastfed infants were also included as a reference group. The primary objective of the study was to demonstrate the equivalence of daily weight gain during the intervention period between the two formula-fed groups. This outcome is discussed in Section 3.3.3. Safety and tolerance were included as secondary objectives, and were assessed by parent-reported gastrointestinal tolerance (regurgitation, vomiting and stool characteristics) and investigator-reported adverse events (AE) at baseline (Visit 1 [V1]) and follow-up visits at 4, 8, 12 and 17 weeks (V2, V3, V4 and V5, respectively). Analysis of safety was performed using the 'All Subjects Treated' dataset, which included all infants with at least one feeding of the study product.

At least one AE was reported in 39.3% of infants in the test group, 31.7% in the control group and 24.6% in the breastfed reference group. The most common AEs were gastrointestinal disorders which were reported in 20.6%, 16.3% and 9.8% of infants in the test, control and reference groups, respectively. Infections and infestations were reported in 15.9%, 16.3% and 13.1% of infants in the test, control and reference groups, respectively. There were no significant differences in the number of total or specific AEs between the two formula-fed groups.

There were no significant differences in the incidence of serious AEs between test and

control formula groups, and all serious AEs were described by the investigator as ‘not related’ or ‘unlikely related’ to the study product.

There were no significant differences in parent-reported gastrointestinal tolerance between the two formula-fed groups.

The study authors concluded that the test formula was safe and well-tolerated in healthy term infants.

3.1.5 Safety assessments by international or national government agencies

The Brazilian Health Regulatory Agency (ANVISA) has assessed and approved the addition of 2′-FL to infant formula and follow-on formula at a concentration of 1 g/L, alone or in combination with GOS and FOS (GOS:FOS ratio 9:1) at 8 g/L. ANVISA concluded that consumption of infant formula and follow-on formula containing 2′-FL, GOS and FOS at these levels is safe (ANVISA 2022).

3.1.6 Summary of the toxicology assessment

FSANZ has previously concluded there are no safety concerns associated with the addition of 2′-FL to IFP and FSFYC at concentrations up to 2.4 g/L. This concentration is within the range of naturally occurring levels of 2′-FL in human milk from the majority of women (0.6 – 7.8 g/L). FSANZ has also previously concluded that there are no safety concerns from the addition of GOS and/or ITF substances to infant formula at concentrations up to 8 g/L for GOS alone or combined with ITF and 3 g/L for ITF.

These conclusions were supported by toxicological studies in laboratory animals and clinical studies in infants which found no adverse effects from the use of these substances.

Clinical studies of infants consuming infant formula containing 2′-FL in combination with scFOS or GOS reviewed for Application A1155 found that these products were well tolerated with no indications of adverse effects.

A newly available clinical study found that consumption of infant formula containing 2′-FL (1 g/L) in combination with a 9:1 ratio of scGOS/lcFOS (8 g/L) was safe and well tolerated.

Given the absence of any identifiable hazard in toxicological and clinical studies with 2′-FL, GOS and/or ITF, including a clinical study of 2′-FL together with scGOS/lcFOS, there are no safety concerns from the addition of 2′-FL in combination with GOS/ITF at the proposed levels.

3.2 Microbiology assessment

3.2.1 Microbiological safety of individual components

A literature review covering the period 2008–2021 (i.e. the period after FSANZ’s finalisation of Proposal P306) confirmed that there were no microbiological safety concerns from the addition of GOS and/or ITF to infant formula products. FSANZ assessments of Applications A1155, A1190 and A1233 (FSANZ 2019a; 2020; 2021; 2022) identified no microbiological safety concerns from the addition of 2′-FL to IFP, and recent studies have not raised any safety concerns.

3.2.2 Microbiological safety of 2′-FL in combination with GOS/ITF

A small number of clinical studies involving infants consuming infant formula containing

various combinations of 2′-FL with GOS and/or ITF indicate that there are no microbiological safety concerns arising.

Marriage et al (2015) and Goehring et al (2016) reported results of a randomised, double-blind growth and tolerance study focused on the effect of 2′-FL in a formula containing GOS. Infants received either of two experimental formulas (EF1, supplemented with 2.2g/L GOS and 0.2g/L 2′-FL; or EF2, supplemented with 1.4g/L GOS and 1.0g/L 2′-FL) or control formula (supplemented with 2.4g/L GOS), and were compared to a breastfed reference group. There were no significant differences between control groups in growth and tolerance measures, including stool frequency and consistency, vomiting and spitting up. No microbiological safety concerns were reported, and there were no significant differences between premature withdrawals from the study between formula groups (Marriage et al, 2015). There were no differences between groups for serum levels of respiratory syncytial virus (RSV), a common cause of respiratory illness in infants (Goehring et al, 2016).

Kajzer et al (2016) provided a preliminary report of findings from a randomised, double-blind tolerance study of a formula containing 2′ FL and scFOS. Infants received either a control formula (EF1, without added oligosaccharides) or an experimental formula (EF2, supplemented with 0.2g/L 2′-FL and 2.0g/L scFOS), and were compared to a human milk-fed reference group. There were no significant differences in stool patterns between groups, apart from the average number of stools per day being significantly greater for the human milk cohort. No difference in tolerance measures was observed between all groups, and no microbiological safety concerns were reported.

Vandenplas et al. (2020) report results of a double-blind, randomised, controlled, multi-country trial where infants received either the test infant formula (supplemented with 26% fermented formula with postbiotics derived from a proprietary fermentation process, 1.0g/L 2′-FL and 8g/L scGOS/lcFOS (9:1)) or control infant formula (standard infant formula containing 8g/L scGOS/lcFOS (9:1)). A breastfed reference group was also included. The primary purpose of this study was anthropometric measurements. Secondary measures included gastrointestinal tolerance and safety (reported adverse events). There was no statistically significant difference between the test and control infant formula groups for regurgitation, vomiting, frequent watery stool or infrequent hard stools at any timepoint. The median stool frequency and frequency of water stool were higher in breastfed infants. No breastfed infants, and very few infants in either the control or test formula groups, had hard stools. Also, there was no statistically significant difference in serious adverse events between the test and control groups. Serious adverse events were classed as unlikely to be related to the study product. This suggests no microbiological safety concerns for infants consuming infant formula containing combinations of 2′-FL and scGOS/lcFOS (9:1).

FSANZ concludes that the limited evidence available from these studies raises no potential microbiological safety concerns from a combination of 2'-FL, GOS and/or ITF in infant formula products at the levels proposed by the Applicant.

3.3 Nutrition Assessment

The objective of the nutrition assessment is to determine the effect of the addition of 2′-FL in combination with GOS and/or ITF to IFP on infant growth.

3.3.1 Previous FSANZ assessments of 2′-FL

FSANZ assessed the effects of 2′-FL on infant growth in A1190 *2′-FL to infant formula and other products* (FSANZ 2021). In addition, FSANZ assessed the effects of 2′-FL in combination with LNnT in IFP on infant growth in Application A1155 *2′-FL and LNnT in infant formula and other products* (FSANZ 2019; FSANZ 2020).

In A1155 FSANZ considered three infant cohort studies (Sprenger et al. 2017; Larsson et al. 2019; Lagström et al. 2020) and five clinical trials in infants (Marriage et al. 2015; Kajzer et al. 2016; Puccio et al. 2017; Storm et al. 2019; Román et al. 2020). The assessment concluded the addition of 2′-FL to infant formula products is not expected to change the growth profiles of infants and young children, at the concentrations typically observed in human milk.

FSANZ notes two of the studies that were assessed as part of A1155 included a combination of two of the relevant oligosaccharides: (Kajzer et al. 2016: 0.2 g/L 2′-FL and 2 g/L scFOS; Marriage et al. 2015: 0.2 g/L 2′-FL and 2.2 g/L GOS, 1.0 g/L 2′-FL and 1.4 g/L GOS). Marriage (2015) reported no significant differences between the two IF or breastfed reference groups for weight, length or head circumference during the four month study period. Kajzer et al. (2016) also reported no significant difference in anthropometric data between groups following consumption of test formula for 35 days, however FSANZ considered the study was not of adequate duration and therefore was not considered in the body evidence.

In A1190 FSANZ assessed four additional publications that studied the effect of infant formula products that contained added 2′-FL on infant growth (Reverri et al. 2018; Berger et al. 2020; Leung et al. 2020; Ramirez-Farias et al. 2021). Following consideration of the additional literature, FSANZ maintained the conclusion that, compared to control formula, no difference in growth was observed in infants fed formula with added 2′-FL.

3.3.2 Previous FSANZ assessments of GOS/ITF

FSANZ assessed the effects of inulin-derived substances, FOS and GOS in P306 – *Addition of Inulin/FOS & GOS to food* (FSANZ 2008a). It was concluded the addition of a total level of 8 g/L of inulin-derived substances and GOS, alone or combined, at any ratio in IFP is unlikely to pose a risk to young infants. FSANZ based that conclusion on data from clinical trials which investigated formulas supplemented with up to 10 g/L of inulin-derived substances and/or GOS to infants, without evidence of harm, although the majority of studies assessed formula supplemented with GOS and inulin-derived substances at 4-8 g/L.

Following a request by the Australia and New Zealand Ministerial Forum on Food Regulation for a review of the approval decision for P306, FSANZ re-assessed the evidence and concluded that a maximum of 8 g/L of IDS added to infant formula products is unlikely to pose a risk to the growth and development of infants fed this formula from birth onwards[5]. Addition of GOS was permitted to a maximum level of 8 g/L and inulin-derived substances were permitted to a maximum level of 3 g/L, as this met the needs of manufacturers (FSANZ 2008b). A combination of IDS and GOS was permitted at a total level of 8 g/L, with no more than 3 g/L of IDS permitted.

In A1055 *Short-chain fructo-oligosaccharides,* FSANZ considered the effects of scFOS on infant growth (FSANZ 2013a). As part of that assessment FSANZ sought advice from the Infant and Child Health Scientific Advisory Group[6] (ICHSAG) on the results of two published studies (Euler et al. 2005; Bettler and Euler 2006) and four unpublished studies (Malacaman et al. 1993; Pickering et al. 1993; Merritt et al. 2005; Imeokparia and Lasekan 2009). The ICHSAG concluded that scFOS had no discernible effect on infant growth patterns. FSANZ

[5] The conclusions in the review were based on the expert opinion of Professor John Cummings, Emeritus Professor of Experimental Gastroenterology, University of Dundee, Scotland, who considered that the evidence indicated no significant differences in terms of growth, weight gain and food intake between infants fed either 8 g/L of IDS or those fed 8 g/L of GOS and IDS in a ratio of 9:1. The omission of the term GOS from the conclusion was an editorial error.

[6] The Infant and Child Health Scientific Advisory Group (ICHSAG) is an independent, external panel of experts that provides advice to FSANZ on issues relating to paediatric growth and development, including nutrition and gastrointestinal health.

concluded that the addition of scFOS to IFP, infant foods and formulated special foods for young children, at the same level at which IDS were permitted, was unlikely to cause adverse physiological effects in the healthy target populations, and the consumption of scFOS-supplemented formula supports normal growth in small infants.

3.3.3 Current assessment

The Applicant provided one infant study that investigated the effect of the combination of 2′-FL, lcFOS and scGOS in infant formula on growth (Vandenplas et al. 2020). Additional studies provided by the Applicant were assessed by FSANZ in previous applications. FSANZ undertook a literature search in Pubmed and no additional relevant studies were identified (Appendix 1).

Vandenplas et al. (2020) studied the effect on growth, safety and tolerance of infant formula containing lcFOS/scGOS and 2′-FL in infants. Some details of the study were previously discussed in Section 3.1.4 and Section 3.2.2 and additional information is provided below. A double-blind, block-randomised, controlled, parallel, multicentre study was undertaken in 192 healthy infants aged up to two weeks in Belgium (3 centres), Hungary (3 centres), Poland (7 centres), Spain (7 centres) and Ukraine (4 centres). Fully breastfeed infants (n = 58) were included as a reference group. Inclusion criteria included healthy infants aged up to 14 days; a gestational age of between 37 weeks and 41 weeks and 6 days; birth weight between 10th and 90th percentile for gestational age and sex; a head circumference at birth within 2 standard deviations of WHO growth standards for age and sex, and an absence of congenital illness or disease that could interfere with study parameters. Infants that had any special dietary requirements or were involved in any other studies were excluded. Breastfed infants were excluded if their mother suffered from any significant medical conditions that may interfere with the study outcomes or were involved in any other intervention trials.

Infants of parents who autonomously decided to formula feed were randomised into blocks of 4, stratified by study site and sex and received either test or control infant formula until 17 weeks of age. The test and control formulas were complete cows' milk based and contained 13 g/L protein, 73 g/L carbohydrate, 34 g/L lipid and 660 Kcal/L. The test formula contained 26% fermented formula with postbiotics derived from the Lactofidus™ fermentation process (with 3′-GL), which involved the addition of *Bifidobacterium breve* C50 and *Streptococcus thermophiles* 065, followed by mild heat treatment (temperature and time not stated). In addition, 0.8 g/100 mL scGOS/lcFOS (9:1), 0.1 g/100 mL 2′-FL, and anhydrous milk fat[7] (49.8% of total fat) were added to the test formula. The control formula was commercially available infant formula containing the same scGOS/lcFOS combination, without 2′-FL, postbiotics or milk fat. Infants were fed *ad libitum* with only the study product during the entire intervention period. Breastfed babies were also fed *ad libitum*.

The primary outcome was equivalence in daily weight gain between infant formula groups from baseline (median age 10 days) to 17 weeks of age. A two-one-sided test was used to determine equivalence in daily mean weight gain between the randomised groups, with a required sample size of 192 infants for $\alpha = 0.05$, power = 0.8 and dropout rate = 25%. Infant weight gain was modelled with a parametric growth curve mixed model with a quadratic function of time. The *a priori* assumptions included a margin of equivalence of ± 3 g/day and an equal within-group standard deviation of 6.1 g in weight gain over the testing period in test and control groups. Secondary outcomes included increased head circumference and length, z-scores for the corresponding anthropometric measurements, parent-reported gastrointestinal tolerance (regurgitation, vomiting, stool characteristics), and investigator-reported adverse events (Section 3.1.4 and Section 3.2.2). Data were collected at baseline, 4

[7] The addition of anhydrous milk fat resulted in a three-fold increase in sn-2 palmitic acid compared to control formula. The authors reported that the addition of sn-2 palmitic acid to infant formula has previously been shown to reduce the formation of insoluble soaps that result in stool softening.

weeks, 8 weeks, 12 weeks and 17 weeks of age. Per-protocol population analysis was undertaken in infants that met all the eligibility criteria including undertaking at least one post-baseline weight measurement and consuming study product exclusively or exclusive breastfeeding for the reference group. Anthropometric z-scores were calculated using a macro provided by World Health Organization (WHO 2006) and analysed using a mixed model for repeated measures.

A total of 215 infants were randomised, and 85 infants in the test formula group and 81 infants in the control group completed the study per-protocol. A similar dropout rate was observed between groups, 16% and 17% respectively. For the breastfed reference group, 50 infants completed the study, with a dropout rate of 8%. No statistically significant difference in infant formula intake was observed between the test and control groups at any time point.

Mean weight and length at each measured timepoint are provided in Table 1. The estimated mean daily weight gain over the study period was 31.0 ± 0.59 (SE), 31.08 ± 0.6 and 28.3 ± 0.79 g/day in the test, control and breastfed reference groups respectively. The difference in estimated mean daily weight gain between test and control groups was -0.08 ± 0.84 g/day [95% CI: -1.47, 1.31]. Length and head circumference gain were similar across the three groups (Table 2).

WHO Growth Standard weight-for-age z-scores were within one standard deviation between groups, which is considered to be within the normal range (WHO 2008). Weight-for-age, length-for-age and head circumference-for-age z-scores for test, control and breastfed reference groups at 17 weeks are provided in Table 2.

Table 1 ***Mean infant weight (g) and length (cm) from baseline (age 0-2 weeks) to study completion (age 17 weeks) in per-protocol test and control populations and breastfed reference groups (Vandenplas et al. 2020)***

	Test		**Control**		**Breastfed**	
	Female	**Male**	**Female**	**Male**	**Female**	**Male**
Age 0-2 weeks						
n	54	47	49	46	31	27
Weight (mean (SD))	3313 (418)	3608 (439)	3322 (314)	3405 (396)	3400 (300)	3460 (375)
Length (mean (SD))	51.6 (2.9)	52.8 (2.7)	51.9 (2.7)	52.3 (2.6)	51.3 (2.4)	52.3 (2.3)
Age 4 weeks						
n	52	45	47	42	28	27
Weight (mean (SD))	4108 (457)	4482 (388)	4110 (371)	4254 (450)	4045 (353)	4252 (420)
Length (mean (SD))	54.2 (2.4)	55.3 (2.1)	54.4 (2.3)	54.9 (2.5)	53.7 (2.3)	54.6 (2.5)
Age 8 weeks						
n	48	41	43	41	27	26
Weight (mean (SD))	5023 (576)	5469 (427)	5011 (487)	5306 (569)	4849 (452)	5293 (518)
Length (mean (SD))	57.2 (2.2)	58.4 (2.6)	56.9 (2.0)	58.0 (2.3)	56.5 (2.1)	57.8 (2.6)
Age 12 weeks						
n	46	40	42	40	25	26
Weight (mean (SD))	5910 (714)	6287 (469)	5810 (627)	6215 (607)	5566 (566)	6092 (572)
Length (mean (SD))	59.9 (2.4)	61.4 (2.4)	59.5 (2.1)	60.9 (2.6)	59.5 (2.3)	60.6 (2.9)
Age 17 weeks						
n	45	40	42	39	25	25
Weight (mean (SD))	6707 (839)	7119 (596)	6631 (697)	7065 (718)	6206 (703)	6890 (577)
Length (mean (SD))	62.8 (2.3)	64.3 (2.2)	62.6 (1.9)	63.7 (2.6)	62.1 (2.5)	63.8 (2.9)

Table 2 *Gain in weight, length and head circumference from baseline to 17 weeks of age, and estimated mean (± 95% confidence interval) WHO Growth Standard z-scores weight-for-age, length-for-age and head circumference-for-age in per-protocol population and breastfed reference groups (Vandenplas et al. 2020)*

	Test (n = 101)		Control (n = 95)		Breastfed (n = 58)	
	Mean (SE)	**95% CI**	**Mean (SE)**	**95% CI**	**Mean (SE)**	**95% CI**
Weight gain (g/day)	31.0 (0.59)	29.8, 32.2	31.1 (0.60)	29.9, 32.3	28.3 (0.79)	26.7, 29.9
Length gain (cm/day)	0.10 (0.0)	0.10, 0.10	0.10 (0.0)	0.09, 0.10	0.1 (0.0)	0.10, 0.11
Head circumference gain (cm/day)	0.06 (0.0)	0.06, 0.06	0.06 (0.0)	0.06, 0.06	0.06 (0.0)	0.06, 0.06
Weight-for-age z-score	0.11	-0.06, 0.28	0.16	0, 0.33	-0.23	-0.45, 0
Length-for-age z-score	0.13	-0.05, 0.32	0.07	-0.12, 0.25	0.06	-0.19, 0.3
Head circumference for-age z-score	0.57	0.38, 0.76	0.57	0.38, 0.76	0.32	0.07, 0.56

The authors concluded that equivalence in daily weight gain from baseline to 17 weeks in infants receiving test and control formula was achieved. Daily weight gain in breastfed infants was also within the margin of equivalence for both test and control infant formula groups.

FSANZ notes some limitations in the study design including the absence of an intention-to-treat analysis and differences in the composition between test and control formulas, (in addition to 2′-FL), including levels of specific lipids (Table 3), although energy content was the same.

Table 3 *Composition of test and control infant formulas (Vandenplas et al. 2020)*

		Test IF (per 100 mL)	**Control IF (per 100 mL)**
Energy	kCal	66	66
Carbohydrates	g	7.3	7.3
scGOS/lcFOS	g	0.8	0.8
2′-FL	g	0.1	0
3′-GL	mg	15	0
Protein	g	1.3	1.3
Whey	g	0.7	0.8
Casein	g	0.7	0.5
Fat	g	3.4	3.4
Vegetable oil	g	1.6	3.3
Dairy lipids	g	1.6	0.1
Saturates	g	1.7	1.5
Palmitic acid	mg	593	581
Sn-2 palmitic acid	mg	202	66.9
Monosaturates	g	1.1	1.3
Polyunsaturates	g	0.6	0.6
Linoleic acid (LA)	mg	448	445
α-Linolenic acid (ALA)	mg	54.9	82
LA:ALA	ratio	8.15	5.40
Arachidonic acid	mg	16.5	11
Docosahexaenoic acid	mg	16.5	10

3.3.4 Key findings of the nutrition assessment

FSANZ has previously assessed the effect of the addition of 2′-FL to IFP on infant growth. The body of evidence included infant cohort and clinical studies, with one suitably designed study that tested IF with two combinations of 2′-FL and GOS finding no significant difference among IF and breastfed reference groups for weight, length or head circumference during the four month study period. It was concluded that 2′-FL should not affect growth at levels normally found in human milk.

In addition, FSANZ has previously assessed the addition of a total level of 8 g/L of inulin-derived substances and GOS, alone or combined, at any ratio in IFP. It was concluded that a maximum of 8 g/L of IDS in IF products is unlikely to pose a risk to the growth and development of infants from birth onwards.

A randomised controlled trial considered in the present assessment found that infant formula containing a combination of 2′-FL (1 g/L), scGOS and lcFOS (9:1, 8 g/L) did not affect growth compared to infant formula containing scGOS and lcFOS (9:1, 8 g/L) without 2′-FL, or to a breastfed reference group, although some limitations were noted in the study design.

FSANZ concludes that, based on the available evidence, no difference in growth is likely to occur in infants fed IFP that contain 2′-FL, scGOS and/or lcFOS at previously permitted levels.

3.4 Dietary intake assessment

3.4.1 Objective

The objective of this dietary intake assessment is to estimate the dietary intake of 2′-FL in combination with GOS and/or ITF from the proposed addition to infant formula and follow-on formula. Infant formula is specified in the Code as being applicable for infants 0-6 months and follow-on formula from 6-12 months.

3.4.2 Previous FSANZ dietary intake assessments of 2′-FL and GOS and/or ITF

3.4.2.1 2'-FL

FSANZ has previously assessed the dietary intake of 2′-FL under A1155 and A1190 (FSANZ 2019, FSANZ 2021). In the dietary intake assessment for A1155 the estimated dietary intakes of 2′-FL_{micro} were similar to the estimated intakes of 2′-FL_{human}. Mean intakes were 2.1 g/day for 3 month old infants and 1.4 g/day for 9 month old infants. This was due to the maximum use level of 2′-FL_{micro} in infant and follow-on formula (2.4 g/L) being similar to the mean concentration of 2′-FL_{human} for human milk (secretors) (FSANZ 2019).

In the dietary intake assessment for A1190, FSANZ undertook a literature search for concentration data for 2′-FL in human milk published since the assessment of A1155. A number of relevant studies were identified, with the range of concentrations reported for secretors (0.9 g/L to 4.0 g/L) falling within a similar range to those reported in previously reviewed studies. It was concluded that an additional dietary intake assessment was not required for A1190 (FSANZ 2021).

3.4.2.2 GOS and/or ITF

For P306 FSANZ conducted a dietary intake assessment of inulin-derived substances and/or GOS in special purpose foods for infants and young children at a maximum use level of 8.0 g/L when added separately or in combination (FSANZ 2008a). The estimated mean

intake of inulin derived substances in combination with GOS was 6 g/day for 3 month old infants and 9 g/day for 9 month old infants (FSANZ 2008a).

3.4.3 Approach to estimating dietary intakes of 2′-FL and GOS and/or ITF

Dietary intake assessments require data on the concentrations of the chemical of interest in the foods requested, as well as any naturally-occurring sources and any current permissions for additions to food; and consumption data for the foods which are usually those collected through a national nutrition survey. The dietary intakes of 2′-FL and GOS and/or ITF for this assessment were estimated using: (1) the maximum use levels of 2′-FL and GOS and/or ITF in the Code; and (2) model diets for infants aged 3 months and 9 months.

Dietary intakes of total HMOs from human milk were also estimated for comparative purposes.

3.4.4 Dietary intake assessment methodology

3.4.4.1 Consumption data used

The hazard identification and characterisation did not identify any population sub-groups for which there were specific safety considerations in relation to the combined intake of 2′-FL and GOS and/or ITF. The population groups that are used for the dietary intake assessment are:

- Infants aged 3 months – representing exclusively formula-fed / breastfed infants
- Infants aged 9 months – representing infants who consume food as well as follow-on formula or human milk.

Model diets were used for the population groups 3 months and 9 months, to represent the consumption of infant formula or follow-on formula (where appropriate) for these groups. This was because food consumption data for individuals in this age group were not included in the most recent nationally representative nutrition surveys in Australia and New Zealand. The model diet for 3 month olds includes only consumption of infant formula. Consumption of solid foods and other beverages are also included in the 9 month old diet. How the model diets were constructed is outlined in Appendix 2.

3.4.4.2 Concentrations of 2'-FL and GOS and/or ITF

Proposed concentrations of 2'-FL and GOS and/or ITF in infant formula and follow-on formula

The food categories requested in the application to contain 2′-FL in combination with GOS and/or ITF and the proposed maximum use levels (in g/L) are listed in Table 4.

Table 4 *Proposed maximum use levels of 2'-FLand GOS and/or ITF in foods, from the Application*

Food	Maximum proposed use level			
	2′-FL		GOS and/or ITF	
	(g/L)	(g/kg)	(g/L)	(g/kg)
Infant formula (as prepared or ready-to-feed)	2.4	2.3	8.0	7.6
Follow-on formula (as prepared or ready-to-feed)	2.4	2.3	8.0	7.6

Note: 1 litre of infant formula and follow-on formula is equal to 1,050 grams.

Concentrations of 2'-FL and GOS and/or ITF in infant formula and follow-on formula used in the dietary intake assessment

The concentrations used in the dietary intake assessment were the maximum permitted levels from the Code. The permissions for addition of substances to infant formula and follow-on formula in the Code (Standard 2.9.1 and Schedule 29) are based on mg/100 kJ units. The maximum levels in the Code for 2′-FL and GOS and/or ITF in infant formula and follow-on formula are 96 mg/100 kJ and 290 mg/100 kJ respectively. As this Application is for the addition of combined 2′-FL and GOS and/or ITF, a total of 386 mg/100 kJ is used in the dietary intake assessment. The maximum proposed use levels from the Applicant (in Table 4) are equivalent to the maximum permitted levels already in the Code for these individual substances with an adjustment made for the energy content of the formula.

Concentration of oligosaccharides in human milk

The reported concentrations of HMOs have been found to vary with the mother's genetic secretor status, length of gestation, lactation period, and method of analysis (Soyyılmaz et al. 2021, Thurl et al. 2017). The Applicant reported the sum of the mean concentrations of individual HMOs in term human milk of secretor mothers from a systematic review as a total for HMOs of 14.78 g/L (Thurl et al. 2017). FSANZ conducted a review of data from the literature which supports the mean concentration provided by Applicant. This concentration of HMOs is greater than the combined maximum use level of 2′-FL and GOS and/or ITF (10.4 g/L) as proposed in this Application.

Concentrations of 2'-FL in domestic mammalian milks

Infant formula and follow-on formula are made with domestic mammalian milk bases, particularly cow milk and goat milk. As the milk itself and foods made from the milks could be consumed by Australian and New Zealand infants, the dietary intake assessment for A1155 assessed the sources of naturally occurring 2′-FL from mammalian milks. The assessment identified that the concentrations of 2′-FL in domestic cow and goat milk were very low, and therefore the contribution of these foods to 2′-FL intakes for infants was likely to be minimal (FSANZ 2019). The contribution of these foods to 2′-FL intakes have not been considered in this current assessment.

Concentrations of GOS and ITF in foods

The estimated dietary intake of naturally occurring and added inulin-derived substances and GOS from foods for 9 month old infants was included in the dietary intake assessment for P306. Concentration data were derived from international literature, industry use data and FSANZ analysis, and mapped to the range of foods consumed by Australian 9 month old

infants (FSANZ 2008a).

In this current Application, the Applicant reported Australian analytical data for GOS, FOS and total fructans in a range of foods including cereals, fruits and vegetables. FSANZ reviewed these data (Muir et al. 2009; Muir et al. 2007 and Biesiekierski et al. 2011) and determined that as the concentration data in these analyses were generally lower than those used in the assessment of P306, estimated intakes are likely not to have increased, and thus an assessment of the contribution of GOS and/or ITF from these foods for 9 month old infants was not required.

3.4.4.4 Assumptions and limitations of the dietary intake assessment

The aim of the dietary intake assessment was to make the most realistic estimation of dietary intakes of 2′-FL and GOS and/or ITF combined as possible. However, where significant uncertainties in the data existed, conservative assumptions were generally used to ensure that the estimated dietary intake was not an underestimate of intake.

Assumptions made in the dietary intake assessment included:

- Unless otherwise specified, all foods within a category contain 2′-FL and GOS and/or ITF at the concentrations specified in Table 1 for infant formula and follow-on formula for human milk
- 1 litre of infant formula and follow-on formula equals 1,050 grams
- 1 litre of human milk equals 1,040 grams
- where a food was not included in the intake assessment, it was assumed to contain a zero concentration of 2′-FL and GOS and/or ITF
- there is 100% market penetration of the use of 2′-FL and GOS and/or IFT into the infant formula and follow-on formula
- infants aged 3 months are exclusively infant formula fed
- infants aged 9 months consume follow-on formula
- consumption of foods as outlined in the model diets represent current food consumption amounts for Australian and New Zealand children aged 3 months and 9 months
- there is no contribution to 2′-FL and GOS and/or ITF intakes through foods and beverages other than from infant formula and follow-on formula
- there is no contribution to 2′-FL and GOS and/or ITF intakes through the use of complementary or other medicines.

In addition to the specific assumptions made in relation to this dietary intake assessment, there are a number of limitations associated with the nutrition surveys from which the food consumption data used for the assessment are based. A discussion of these limitations is included in Section 6 of the https://www.foodstandards.gov.au/publications/Pages/Principles-and-Practices-of-Dietary.aspx.

3.4.5 Estimated dietary intakes

3.4.5.1 Estimated dietary intake of HMOs from human milk

When it is assumed that infants aged <12 months are consuming human milk (and no infant formula or follow-on formula), the estimated mean intakes of HMOs from human milk is 10.9 g/day for 3 month old infants and 7.3 g/day for 9 month old infants. Whereas the P90 intake is 21.7 g/day for 3 month old infants and 14.6 g/day for 9 month old infants.

On a grams per kilogram body weight per day basis, the estimated mean and P90 dietary intakes of HMOs from human milk are 1.7 g/kg bw/day and 3.4 g/kg bw/day for 3 month old infants and 0.8 g/kg bw/day and 1.6 g/kg bw/day for 9 month old infants.

Further details are presented in Table 5.

Table 5 *Estimated dietary intakes of human milk oligosaccharides for infants aged 3 months and 9 months consuming human milk*

	Unit	*3 months*	*9 months*
Recommended energy intake[1]	kJ/kg bw/day	343	330
P50 body weight[2]	kg	6.4	8.9
Recommended energy intake	kJ/day	2195	2937
Amount of human milk required to meet 100% energy requirements[3]	g/day	765	n/a
Amount of human milk required to meet 50% energy requirements[3]	g/day	n/a	515
Mean dietary intake of HMOs from human milk[4]	g/day	10.9	7.3
	g/kg bw/day	1.7	0.8
P90 dietary intake of HMOs from human milk[4]	g/day	21.7	14.6
	g/kg bw/day	3.4	1.6

1 United Nations University et al. 2004.
2 World Health Organization 2006.
3 Energy content of human milk is 286 kJ/100 g (FSANZ, 2016).
4 Concentration of HMOs used in calculation is 14.78 g/L (Thurl et al. 2017). 1 L of human milk is equivalent to 1,040 g.

3.4.5.2 Estimated dietary intake of 2'-FL and GOS and/or ITF combined from infant formula

The estimated mean and P90 combined dietary intakes of 2′-FL and GOS and/or ITF from infant formula are 8.5 g/day and 16.9 g/day for 3 month old infants, and from follow-on formula are 5.7 g/day and 11.3 g/day for 9 month old infants.

On a grams per kilogram body weight per day basis, the estimated mean and P90 dietary intakes of 2′-FL and GOS and/or ITF from infant formula are 1.3 g/kg bw/day and 2.6 g/kg bw/day for 3 month old infants, and from follow-on formula are 0.6 g/kg bw/day and 1.3 g/kg bw/day for 9 month old infants (see Table 6).

Table 6 *Estimated mean dietary intake of the combined addition of 2'-FL and GOS and/or ITF in infant and follow-on formula based on maximum permitted concentrations in the Code**

	Unit	3 months[3]	9 months[3]
Recommended energy intake[1]	kJ/kg bw/day	343	330
P50 body weight[2]	kg	6.4	8.9
Recommended energy intake	kJ/day	2195	2937
100% energy requirements[3]	kJ/day	2195	n/a
50% energy requirements[3]	kJ/day	n/a	1469
Mean dietary intake of 2′ FL+ GOS and/or ITF from infant or follow-on formula	g/day	8.5	5.7
	g/kg bw/day	1.3	0.6
P90 dietary intake of 2′ FL+ GOS and/or ITF from infant or follow-on formula	g/day	16.9	11.3
	g/kg bw/day	2.6	1.3

* Equivalent to the maximum permitted use levels requested by the Applicant.
[1] United Nations University et al. 2004.
[2] World Health Organization 2006.
[3] The maximum combined concentration of 2′-FL and GOS and/or ITF in infant formula and follow-on formula is 386 mg/100 kJ.

3.4.6 Conclusion

Based on the maximum permitted concentration levels in the Code (which are equivalent to the maximum use levels proposed by the Applicant), the estimated mean and P90 intakes of 2′-FL combined with GOS and/or ITF from infant formula and follow-on formula range between 5 and 17 g/day. These intakes are less than the estimated mean and P90 intakes of HMOs from human milk.

4 Health effects assessment

4.1 Anti-pathogenic effects

No human intervention studies investigating an anti-pathogenic effect of a combination of 2'FL and GOS and/or ITF were provided by the Applicant or identified by FSANZ. Some indirect evidence for additive or synergistic effects of the two classes of oligosaccharide was provided.

Overbeek et al (2019) provided a preliminary report on the effect of a combination of 2′-FL and scGOS/lcFOS on maturation of human dendritic cells and cytokine production in co-culture with T-cells *in vitro*. They saw a synergistic effect of addition of the oligosaccharides on production of a number of cytokines, and concluded that this would affect the T_H1/T_H2 (T helper cell) balance in favour of a pro-inflammatory, anti-infective immune response. A statistically significant shift towards a higher T_H1/T_H2 ratio was also observed in animal studies involving supplementation of the diets of suckling rats and 6 week old (adult) mice with a combination of 2′ FL and scGOS/lcFOS, implying a contribution of the combination of the oligosaccharides to protective immunity against infections (Azagra-Boronat et al 2018; Xiao et al 2019).

Azagra-Boronat et al (2018) assessed the effect of 2′-FL and scGOS/lcFOS—separately and combined—on rotavirus infection and diarrhoea in suckling rats, and undertook *in vitro* assessment of the ability of the compounds to bind directly to viral particles and block binding of an anti-rotavirus monoclonal antibody. scGOS/lcFOS demonstrated strong binding to rotavirus particles, with 2′-FL contributing a small, but statistically significant, additive blocking effect. This viral particle binding effect is one plausible mechanism by which the

compounds can inhibit viral infection. Additionally, 2′-FL was observed to enhance the intestinal barrier function and to reduce the severity of diarrhoea to a greater degree than scGOS/lcFOS, while an additive effect was observed in reducing the incidence of rotavirus-induced diarrhoea.

The extent to which these results are applicable to human infant immune development and protection against pathogen infection and disease *in vivo* is unclear. However, the results are consistent with effects observed for the individual components.

4.2 Bifidogenic effects

Both 2′-FL and scGOS/lcFOS have been individually demonstrated to have a bifidogenic effect *in vivo* (FSANZ 2019a; Béghlin et al 2021; Holscher et al 2012; Scholtens et al 2008). No human intervention studies investigating a bifidogenic effect of a combination of 2′ FL and GOS/ITF were provided by the Applicant or identified by FSANZ. Some indirect evidence for synergistic effects of the two classes of oligosaccharide was provided.

Goh et al (2019) provided a preliminary report on the effect of a combination of 2′-FL and scGOS/lcFOS on the composition and metabolic activity of infant gut microbiota in a simulator of the human intestinal environment. The simulator was inoculated with a faecal sample from a healthy 3 month old infant that was born via C-section, exclusively breastfed, and had no history of antibiotic usage. Samples were collected from vessels simulating the proximal and distal colons over a 2 week period.

Some short-chain fatty acids (SCFA), such as acetate, propionate and butyrate, have been shown to have antimicrobial and anti-inflammatory effects and have been linked to maintenance of intestinal barrier integrity (Silva et al 2020; Tan et al 2014). The effect of supplementation on SCFA production varied depending on the SCFA assayed (Goh et al 2019). Acetate and propionate production were strongly influenced by scGOS/lcFOS. Supplementation with 2′-FL had no significant effect in the presence or absence of scGOS/lcFOS. In contrast, butyrate production was strongly induced by 2′-FL supplementation, with no additive effect of co-supplementation with scGOS/lcFOS. In the absence of 2′FL, scGOS/lcFOS slightly supressed butyrate production in the distal colon simulator after 11–15 days (Goh et al (2019). Production of isobutyrate was suppressed in the presence of scGOS/lcFOS, with a small but significant additive effect in the presence of 2′-FL (Goh et al 2019). Branched chain fatty acids (BCFA) such as isobutyrate are produced from protein/amino acid fermentation, which has been suggested to have detrimental health effects (Rio-Covian et al 2020).

Analysis of carbohydrate utilisation in the intestinal simulator showed a strong synergistic effect on 2′-FL utilisation when co-supplemented with scGOS/lcFOS. In the absence of scGOS/lcFOS, 2′-FL was not greatly utilised (Goh et al 2019).

The abundance of *Bifidobacterium* species in the proximal colon simulator was strongly increased due to supplementation with scGOS/lcFOS, with little or no effect of 2′-FL alone or when added with scGOS/lcFOS. Similarly, Proteobacteria (which includes several genera of human bacterial pathogens) were strongly suppressed by scGOS/lcFOS, while 2′-FL had no impact on abundance. In contrast, the abundance of *Veillonella*—a genus of bacteria commonly found in the infant gut that utilises lactate and produce propionate—was more strongly influenced by 2′-FL supplementation, with only a small additive effect of co-supplemenation with scGOS/lcFOS (Goh et al 2019).

The results imply a complex interaction between the carbohydrate sources, infant microbiota composition and the profile of SCFA produced. They suggest that the combination of 2′-FL and scGOS/lcFOS has a bifidogenic effect and can alter the microbiome environment to be

less-conducive to the growth and survival of some human pathogenic bacteria.

Van den Elsen et al (2019) showed that supplementation of the diet of mice from weaning (at 3 weeks of age) with a combination of 2′-FL and scGOS/lcFOS greatly increased the abundance of Actinobacteria—a phylum which includes the genus *Bifidobacterium*—in faecal samples collected between 3 and 10 weeks after initiation of dietary supplementation. No comparison was made with the effect of supplementation with 2′-FL or scGOS/lcFOS separately.

The available evidence is insufficient to draw conclusions on how the magnitude of the bifodogenic effect of a combination of 2′-FL and scGOS/lcFOS *in vivo* compares to the effects of the components individually.

4.3 Health effects conclusion

No human intervention studies investigating a bifidogenic or anti-pathogenic health effect of the combination of 2′-FL and GOS and/or ITF were provided by the Applicant or identified by FSANZ.

In vitro and animal studies provide indirect evidence for these health effects, and some indication of mechanisms involved, and the results are consistent with effects observed for the individual components. No evidence analysed implied any antagonistic effects between the individual components. However, the evidence is insufficient to draw conclusions on how the magnitude of the effects due to the combination of 2′-FL and GOS and/or ITF compare to the effects of the individual components. The results imply an additive and/or synergistic effect of a combination of 2′-FL and GOS and/or ITF on some measures. However, it is unclear whether they are applicable to human infants fed formula supplemented with a combination of 2′-FL and GOS and/or ITF.

5 Conclusions of the risk and technical assessment

FSANZ has undertaken a risk and technical assessment of the proposed addition of a combination of 2′-FL with GOS and/or ITF to IFP.

The safety, technological, nutritional impact and beneficial health effects from individual addition of these ingredients to infant formula products have previously been considered (in Applications A1155, A1190 and A1233 for 2′-FL; Proposal P306 for GOS/ITF and A1055 for scFOS). The purpose of the present assessment was to consider the combination of these ingredients. Previous assessment found that 2′-FL is stable, structurally and chemically identical to naturally occurring 2′-FL and free from fermentation derived contaminants (FSANZ 2019). GOS and/or ITF were assessed as not considered to pose a risk to the health and safety of young infants (FSANZ 2008a). New information has been provided to assess the stability of the blended ingredients with FSANZ confirming that the ingredients provide an adequate shelf-life and stability.

FSANZ has previously determined that there are no safety concerns associated with the addition of 2′-FL, GOS and/or ITF to IFP at concentrations up to 2.4 g/L for 2′-FL, 8 g/L for GOS alone or combined with ITF, and 3 g/L for ITF alone. These conclusions were supported by toxicological studies in laboratory animals and clinical studies in infants which found no adverse effects from the use of these substances.

A newly available clinical study reviewed by FSANZ for the present assessment found that consumption of infant formula containing a combination of 2′-FL (1 g/L) with a 9:1 ratio of

scGOS/lcFOS (8 g/L) was safe and well tolerated.

The limited evidence available from human intervention studies raised no potential microbiological safety concerns from a combination of 2'-FL with GOS and/or ITF in infant formula products at the levels proposed by the Applicant.

FSANZ has previously concluded that 2′-FL added to IFP should not affect growth at levels normally found in human milk. In addition, FSANZ has previously assessed the addition of a total level of 8 g/L of GOS and/or ITF, alone or combined at any ratio, in IFP. It was concluded that a maximum of 8 g/L in IF products is unlikely to pose a risk to the growth and development of infants from birth onwards. One additional relevant study was considered for the present assessment. The authors reported no difference in growth in infants that received formula containing a combination of 2′-FL (1 g/L) and scGOS/lcFOS (9:1; 8 g/L), although some limitations in study design were noted.

FSANZ maintains the conclusion that, based on the currently available information, infant formula that contains a combination of 2'FL and GOS/ITF at the requested concentrations is not likely to affect infant growth.

Dietary intakes of 2′-FL in combination with GOS and/or ITF from IFP were estimated for infants using a model diet approach. The estimated mean and 90th percentile (P90) dietary intakes of 2′-FL combined with GOS and/or ITF from infant and follow-on formula ranged between 5 and 17 g/day. These intakes were lower than the estimated mean and P90 intakes of human milk oligosaccharides (HMOs) from human milk.

Given the absence of any identifiable hazard in toxicological and clinical studies with 2′-FL, GOS and/or ITF, alone or in combination, and noting that estimated exposures are lower than those of HMOs from human milk, there are no safety concerns from the addition of 2′-FL in combination with GOS and/or ITF to IFP at the proposed levels.

No human intervention studies investigating a bifidogenic or anti-pathogenic health effect of the combination of GOS and/or ITF with 2′-FL were provided by the Applicant or identified by FSANZ. Results from *in vitro* and animal studies of combinations of 2′-FL and GOS and/or ITF are consistent with beneficial health effects observed for the individual components and provide some indication of mechanisms involved. However, they do not allow any conclusions to be drawn on whether there are any additional benefits arising from supplementation with a combination of 2′-FL and GOS and/or ITF.

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Appendix 1: Search strategy for infant and toddler growth assessment

FOS or inulin type fructans or inulin-type fructans or fructo oligosaccharide or fructo-oligosaccharide or galacto-oligosaccharide or galacto oligosaccharide
AND
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

Search: milk[Title/Abstract] OR breast[Title/Abstract] OR formula[Title/Abstract] Sort by: First Author
AND
Search: Anthropometric[Title/Abstract] OR weight[Title/Abstract] OR growth[Title/Abstract] OR development[Title/Abstract] Sort by: First Author
AND
Search: child[Title/Abstract] OR infant[Title/Abstract] OR baby[Title/Abstract] OR maternal[Title/Abstract] Sort by: First Author
AND
Search: FOS or inulin type fructans or inulin-type fructans or fructo oligosaccharide or fructo-oligosaccharide or galacto-oligosaccharide or galacto oligosaccharide or galactooligosaccharide Sort by: First Author
AND
Search: ((((2′-fucosyllactose) OR (2′ fucosyllactose)) OR (2′-FL)) OR (2′FL)) OR (2′ FL) Sort by: First Author

Appendix 2: How the infant model diets were constructed

Children aged 3 and 9 months

As there are no data available from the 2011-12 Australian National Nutrition and Physical Activity Survey or the 2002 New Zealand National Children's Nutrition Survey for children less than two years of age, model diets were constructed to estimate the combined 2′-FL and GOS and/or ITF intakes for children aged 3 months and 9 months. The same model diets were used for Australia and New Zealand.

As the 3 month and 9 month old infant model diets are based on mean food consumption amounts only, a distribution of food consumption was not available, and hence, a distribution of the combined intake of 2′-FL and GOS and/or ITF was not able to be produced. Therefore, the 90th percentile dietary intake were estimated using the calculation shown in **Error! Reference source not found.**.

Equation 1: 90th percentile dietary exposure calculation for the 3 month, 9 month and 12 month old infant model diets

$$90^{th} \text{ percentile exposure} = \text{mean exposure} \times 2^{*}$$

* (World Health Organization et al.,1985)

The energy content of human milk is required for the calculation of the amount of human milk in the model diets for 3 month and 9 month old infants. AUSNUT 2011-13 (the nutrient dataset for the 2011-12 National Nutrition and Physical Activity Survey (NNPAS)) is the latest nutrient data set published for Australian foods. In this dataset, the energy content of *Milk, human/breast, mature, fluid* is 286 kJ/100 g (FSANZ, 2016). A set of model diets were developed using the AUSNUT energy contents for human milk in the calculation of human milk consumption for 3 month and 9 month old infants as shown in Table 5.Table .

A set of model diets was not established for infants consuming infant formula products for special dietary uses as the energy and/or fluid requirements can vary depending on the medical conditions of the infant. Additionally, the energy content of the various infant formula products for special dietary uses can be variable. The assessment of A1155 included an examination of a range of products on the market, including formulas for premature infants, formulas for use by infants with inborn errors of metabolism, and formulas for use by infants with severe food allergies, which found the range of energy contents was 269 – 415 kJ/100 g. If an infant consuming infant formula products for special dietary uses has similar energy requirements to those used in the model infant diets and their specific formula has a similar energy content to that used in the model diets, then their intake of 2′-FL and GOS and/or ITF is anticipated to be similar to that outlined in the assessment below. If an infant consuming infant formula products for special dietary uses has similar energy requirements to those used in the model infant diets and their specific formula has a higher energy content to that used in the model diets, then their intake of 2′-FL and GOS and/or ITF is anticipated to be similar to or lower than that outlined in the assessment above.

Infants aged 3 months

The recommended energy intake for a three-month-old boy (343 kJ/kg bw/day) (United Nations University et al. 2004) and the 50th percentile weight (6.4 kg) (World Health Organisation 2006) for the same age and sex were used as the basis for the model diet. Boys' weights were used because boys tend to be heavier than girls at the same age and therefore have higher overall energy and food requirements. The entire energy requirement

in the 3 month old infant diet is derived from infant formula or human milk, depending on the assessment. The body weight of 6.4 kg was used to estimate dietary intakes for 3 month old infants on a body weight basis.

Infants aged 9 months

By the age of 9 months, infants are consuming a mixed diet of solids and follow-on formula / human milk. The model diet was constructed based on recommended energy intakes, mean body weight and the proportion of milk and solid foods in the diet for a 9 month old infant. The recommended energy intake for a 9 month old boy (330 kJ/kg bw/day) (United Nations University et al. 2004) and the 50th percentile weight (8.9 kg) (World Health Organisation 2006) for the same age and sex was used as the basis for the model diet. It was assumed that 50% of energy intake was derived from follow-on formula / human milk and 50% from solids and other fluids (Butte et al, 2004; Hitchcock 1986; Pan American Health Organization, 2003). The body weight of 8.9 kg was used to estimate dietary intakes for 9 month old infants on a body weight basis. As noted in the sections discussing concentrations of 2'FL, GOS and ITF in solid foods, the concentrations of these are negligible or lower than previous FSANZ dietary intake assessments and therefore do not need to be considered in the dietary intake assessment for this Application. Therefore, the solid food component of the model diet that is usually included for assessments for 9 month olds, did not need to be included in this case.