# Application to amend the Australia New Zealand Food Standards Code to permit a combination of GOS/ITF and 2'-FL in infant formula products

**Nutricia Australia Pty Ltd (Nutricia) & Chr. Hansen A/S**

**February 2022**

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## ABBREVIATIONS

| Abbreviation | Explanation |
|---|---|
| 2'-FL | 2' – fucosyllactose |
| A1055 | Application A1055 - Short Chain Fructo-oligosaccharides |
| A1155 | Application A1155 – 2'-FL and LNnt in infant formula and other products |
| A1190 | Application A1190 – 2'-FL in infant formula and other products |
| A1233 | Application A1233 - 2'-FL from new GM source for infant formula |
| AE | adverse event |
| ANVISA | Brazilian Health Regulatory Agency |
| AU $ | Australian dollars |
| DFL | difucosyllactose |
| EFSA | European Food Safety Authority |
| EU | European Union |
| FAO | Food and Agriculture Organisation |
| FSANZ | Food Standards Australia New Zealand |
| HMO | human milk oligosaccharide |
| g/d | grams per day |
| g/L | grams per litre |
| GOS | galacto-oligosaccharides |
| GRAS | generally recognized as safe |
| IFP | infant formula products |
| ITF | inulin-type fructans (including FOS) |
| kcal/dL | kilocalories per decilitre |
| lcFOS | long-chain fructo-oligosaccharides |
| LNnt | lacto-N-neotetraose |
| mg/100 kJ | milligrams per 100 kilojoules |
| NHMRC | National Health & Medical Research Council (Australia) |
| NNS | National Nutrition Survey |
| NZ $ | New Zealand dollars |
| P306 | Proposal P306 – Addition of inulin/FOS & GOS to food |
| SAE | serious adverse event |
| scGOS | short-chain galacto-oligosaccharides |
| SFA | Singapore Food Agency |
| USA | United States of America |
| WHO | World Health Organisation |

## INTRODUCTION

Breastfeeding provides the best nutrition for babies, which is why we recognise breast milk as uniquely superior for babies. Breast milk provides many benefits. The WHO's global public health recommendation calls for exclusive breast feeding for the first six months of life, followed by the introduction of safe and appropriate complementary foods, whilst encouraging breast feeding up to two years of age. However, for infants that cannot or are not fed breast milk, commercial infant formulas are recommended to be used as an alternative to breast milk until twelve months of age (National Health & Medical Research Council (Australia)) (NHMRC 2012). Infant formulas are manufactured with ingredients that attempt to resemble breast milk components as closely as possible, not only in terms of composition but also in relation to the nutritional needs and functional benefits to meet the growth and development requirements of infants.

Human breast milk contains many bioactive compounds including human milk oligosaccharides (HMOs), immune cells, bacteria and bacterial metabolites that support normal infant growth and development by promoting a healthy gut microbiota which is significant during infancy. HMOs are the third largest solid component found in breast milk – there are over 200 different structures of oligosaccharides including short and long chain structures at levels of around 10-15 g/L in mature breast milk which act together to support the four main components of the immune system - mechanical, chemical, biological and immunological defences (Thurl et al. 2017).

A number of infant formula products are manufactured to contain oligosaccharides to provide formula-fed infants with the benefits of HMOs present in breast milk. Galacto-oligosaccharides (GOS) and inulin-type fructans (ITF) and have been added to infant formula products for approximately 20 years. FSANZ clarified the regulatory position in Australia and New Zealand with Proposal P306 (in 2008), permitting the addition of ITF (including long chain fructo-oligosaccharides derived from inulin (referred to by Nutricia and in this application as lcFOS)) and short chain galacto-oligosaccharides (scGOS) to infant formula products (IFP). Nutricia has been adding its patented scGOS/lcFOS mixture to IFP marketed in Australia and New Zealand since 2008 and internationally since 2000 at a level of 8 g/L at a patented ratio of scGOS/lcFOS of 9:1 (7.2 g/L GOS and 0.8 g/L FOS).

The addition of other oligosaccharides to infant formula products has recently been permitted by FSANZ. Two applications (A1155 and A1190) have been assessed by FSANZ, resulting in permission in Standard 2.9.1 – Infant formula products – being granted for the addition of 2'-fucosyllactose (2'-FL) at a level of up to 2.4 g/L. 2'-FL is an HMO identical ingredient sourced by enzymatic fermentation by genetically modified microorganisms (*E. coli* sp). The second of these applications was originally submitted by Jennewein Biotechnologie GmbH, a company that has since been acquired by Chr. Hansen A/S (Chr. Hansen). Chr. Hansen formally notified FSANZ that it had become the applicant for A1190. Nutricia has partnered in this application with Chr. Hansen to seek permission for the voluntary addition of Chr. Hansen 2'-FL to IFP that contain a mixture of GOS and ITF (for Nutricia, specifically scGOS/lcFOS (9:1 ratio) mixture). Standard 2.9.1 currently permits only GOS/ITF or a specified 2'-FL to be added to infant formula products, not a combination of GOS/ITF and 2'-FL ingredients (section 2.9.1—7(2)).

Therefore, this application, jointly submitted by Nutricia and Chr. Hansen, requests permission for the voluntary addition of a combination of 2'-FL and GOS/ITF to IFP. The application relates only to infant formula and follow-on formula for infants aged up to twelve months, Including infant formula products for special dietary use. In addition, the application relates only to permitting a combination of 2'-FL and GOS/ITF to infant formula products; and does not relate to LNnt, which is an oligosaccharide that is also permitted to be added to IFP in the Code.

As noted above, the safety and benefit of adding 2'-FL and GOS/ITF to IFP has been described and assessed in detail in separate processes by FSANZ. In recognition of these previous comprehensive assessments by FSANZ (including A609, A309, A1055, A1155 and A1190), this application does not focus on the safety and benefit of adding 2'-FL or GOS/IFT to IFP in isolation, although it does update current information on safety and benefit to add to the comprehensive FSANZ assessments. Rather, the application focuses on the safety and benefit of adding a combination of Chr. Hansen's 2'-FL and GOS/IFT to IFP. The safety of the combination of these ingredients is addressed in Section C and the benefit is addressed in Section E.

Pre-application discussions with FSANZ identified the total oligosaccharide load resulting from the intended use of 2'-FL in combination with GOS/ITF in IFP should be considered in the context of the oligosaccharide load in human breast milk. The oligosaccharide content of breast milk has been reported to vary between individuals; however, the average content of HMOs has been reported by Thurl et al. (2017) to be 15 g/L in mature breast milk and up to 20 g/L in colostrum. The addition of 2'-FL at 2.4 g/L and GOS/ITF at 8 g/L will provide a total of 10.4 g/L of added oligosaccharides in infant formula products, which is lower than the reference values for mature breast milk. The addition of 2'-FL at 2.4g/L is based on the permitted level in Standard 2.9.1 as a result of A1155 and A1190.

This application demonstrates that the addition of 2'-FL at up to 2.4 g/L and GOS/ITF at up to 8 g/L to IFP is safe, well-tolerated and provides formula fed infants with normal growth and developmental support and benefits similar to breastfed infants.

## GENERAL REQUIREMENTS

### 1 Applicant Details

| Applicants | Nutricia Australia Pty Ltd<br><br>Chr. Hansen A/S |
|---|---|
| [redacted] | [redacted] |
| [redacted] | [redacted] |
| [redacted] | [redacted] |
| [redacted] | [redacted] |
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| [redacted] | [redacted] |
| [redacted] | [redacted] |

## 2 Purpose of the application

*(addressing section 3.1.1.C of the FSANZ Application Handbook)*

The application requests amendment of the Australia New Zealand Food Standards Code (the Code) to permit the addition of 2'-fucosyllactose (2'-FL) marketed by Chr. Hansen to infant formula products that also contain GOS/ITF (including a specific 9:1 ratio of galacto-oligosaccharide (scGOS) and inulin-type fructan (fructo-oligosaccharide (lcFOS)) as manufactured by Nutricia). Each of these ingredients are permitted to be added to infant formula products, however section 2.9.1—7(2) of the Code does not permit 2'-FL to be added to infant formula products which contain added inulin-type fructans (ITF) and/or GOS (reproduced below).

**2.9.1—7 Restriction on addition to infant formula product of inulin-type fructans and galacto-oligosaccharides**

(2) An infant formula product to which an inulin-type fructan or a galacto-oligosaccharide is added must not contain any of the following added substances:

(a) 2′-O-fucosyllactose; or

(b) a combination of 2′-O-fucosyllactose and lacto-N-neotetraose.

Nutricia uses a patented scGOS/lcFOS mixture that is currently permitted to be added to infant formula products at 8 g/L at a ratio of 9:1. Nutricia intends to also add 2'-FL to infant formula products containing the scGOS/lcFOS mixture at the currently permitted level of 2.4 g/L. Nutricia does not intend to add lacto-N-neotetraose (LNnt) to these infant formula products. Therefore, this application requests only permission to use a combination of GOS/ITF (including Nutricia's scGOS/lcFOS (9:1 ratio)) and Chr. Hansen's 2'-FL at levels of 8 g/L and 2.4 g/L respectively in infant formula products.

Therefore, the purpose of the application is to vary the prohibition under 2.9.1—7(2) to remove paragraph (a). This will permit the addition of 2'-FL to infant formula products that also contain added GOS/IFT. For clarity, this application does not request any change to the existing paragraph (b) of section 2.9.1—7 because Nutricia and Chr. Hansen are not requesting permission to add a combination of 2'-FL and LNnt to infant formula products that also contain added GOS/IFT.

Nutricia has partnered with Chr. Hansen to source its 2'-FL to add to its Nutricia infant formula products that contain a scGOS/lcFOS (9:1 ratio) mixture. Chr. Hansen acquired Jennewein Biotechnologie GmbH, which submitted an original application to FSANZ, Application A1190 (FSANZ 2021a), requesting permission to add its brand of 2'-FL (Jennewein 2'-FL) to infant formula products. Chr. Hansen formally advised FSANZ it was the applicant after acquiring Jennewein. FSANZ has completed the assessment of that application to approve the addition of Chr. Hansen 2'-FL to infant formula products. The FSANZ Board approval is currently with the Food Ministers Meeting for approval, and subsequent gazettal in the Code is expected in January 2022.

The safety, tolerance and benefit of the addition of Chr. Hansen 2'-FL to infant formula products has been assessed and approved by FSANZ during the assessment of Application A1190. The safety of addition of GOS and ITF substances to infant formula products has also been assessed and approved by FSANZ during Proposal P306 (FSANZ 2008), A1055 (FSANZ 2013) and A1155 (FSANZ 2019). The purpose of adding ITF substances and GOS to infant formula products was also addressed in P306 and A1155. Given the individual ingredients, 2'-FL and GOS/ITF have previously been assessed by FSANZ and permitted to be added to infant formula products, the focus of this application is on the addition of a combination of 2'-FL and GOS/ITF. Therefore, the information provided in support of this application focusses on the safety, tolerance and benefit of adding a combination of Chr. Hansen 2'-FL and GOS/ITF (specifically Nutricia scGOS/lcFOS (9:1 ratio)) to infant formula products.

### 2.1 Clarification on definitional issues

GOS and ITF are not considered to be substances that are used as a nutritive substance in the context of the Code; both are excluded from the Code's definition of *used as a nutritive substance* and are permitted to be present as ingredients in infant formula, subject to the restrictions in section 2.9.1--7(1). However, the Code does consider 2'-FL to be a substance that is used as a nutritive substance in the context of addition to infant formula. This application does not seek to amend the current status of GOS/ITF and 2'-FL in relation to the Code's definition of *used as a nutritive substance.* However, the application has been structured in accordance with the Application Handbook's requirements for nutritive substances; to ensure information on both 2'-FL and GOS/ITF is presented in a consistent manner.

## 3 Justification for the application

### 3.1 Need for the proposed change

*(addressing section 3.1.1.D.(a) of the FSANZ Application Handbook)*

The combination of an added GOS/ITF mixture and 2'-FL in infant formula products is not currently permitted by section 2.9.1—7(2)(a) of the Code. Amendment of this section is therefore required before 2'-FL and GOS/ITF can be added in combination to infant formula products in Australia and New Zealand.

Infant formula products that contain 2'-FL are currently approved for use in 71 countries, including Australia and New Zealand. This includes IFP that also contain GOS/ITF. The proposed change adds additional support for innovation and product improvement for companies within Australia and New Zealand to remain competitive in global markets through export, whilst providing these products to the domestic market. It also allows the import of infant formula products, that includes special dietary use products, manufactured in other countries.

The proposed change to allow voluntary combined addition of GOS/ITF mixture and 2'-FL in infant formula products provides nutritional and developmental benefits to infants that cannot or are not fed breastmilk, to a larger extent than the single ingredients and is therefore another step closer to the complexity of human milk oligosaccharides composition and developmental outcomes of human milk.

## 3.2 Regulatory impact information

*(addressing section 3.1.1.D.(b) of the FSANZ Application Handbook)*

3.1.1 Costs and benefits of the application

*a) Consumers*

Consumers will benefit from the continued innovation and investment in infant formula by industry for infants that cannot or are not fed breast milk. Commercial infant formulas are recommended to be used as the only alternative to breast milk until twelve months of age (NHMRC 2012). Infant formulas are manufactured with ingredients that attempt to resemble breast milk components as closely as possible, not only in terms of composition but also in relation to the normal growth and development of infants fed with breast milk.

In the 2020 review of the decision of A1155, FSANZ provided justification for benefits of adding HMOs to infant formula that took into consideration "that a 'health outcome' for a voluntary addition to infant formula products should be considered in the context of shifting outcomes of formula-fed infants closer to those of breastfed infants, recognising that not all infants are able to be breastfed. FSANZ recognises that infant formula products will never provide the same benefits as breastfeeding or human milk. However, infants who are not breastfed should not be prevented from having access to products that more closely resemble human milk." (FSANZ 2020).

FSANZ reiterated the justification for benefits of adding HMOs to infant formula products in the A1190 Approval Report (FSANZ 2021a).

Products containing added HMOs and GOS/ITF are available internationally. A comprehensive list of countries in which both Nutricia and other branded products containing both ingredients are marketed is provided in section D.5.

*b) Industry*

Approval will facilitate trading opportunities and supports innovation of infant formula products to be manufactured in Australia and New Zealand and sold domestically in both countries as well as exported to international markets.

Approval would allow Australian and New Zealand manufacturing companies to compete on a level playing field and not be commercially disadvantaged with countries within the EU (Germany, The Netherlands, Ireland, Switzerland), USA, China and India that manufacture products containing these ingredients for different export markets world-wide.

It will minimise trade restrictions for imported product that contain these ingredients from countries where these ingredients are permitted to be added.

Finally, it supports manufacturing industry in Australia and New Zealand, to allow manufacturers to manufacture these products to grow domestic market share and export opportunities, with subsequent investment in the local dairy industry, capital expenditure in manufacturing sites, job retention and growth.

In the reconsideration of the A1155 decision, FSANZ was provided information from ABARES and New Zealand MPI valuing infant formula exports at "more than NZ $1.7 billion in 2019, and Australia's 2018 exports...approximately AU $789 million." (FSANZ 2020).

*c) Government*

Allows governments in Australia and New Zealand to have consistent regulation and standards with other countries. Supports international consistency and considered harmonisation with international food standards.

Allows product for export, manufactured in New Zealand, to be exported without seeking an exemption under the Ministry for Primary Industries Food Notice – Food for Export – Exemptions from Domestic Compositional Requirements No. 6 2020, which is an added time and cost impost for manufacturing companies located in New Zealand.

3.1.2 Impact on international trade

Promotes international trade and improves competitiveness of products, manufactured in Australia and New Zealand, for sale in international markets.

Whilst assisting international trade access, as FSANZ noted in the A1155 Approval Report, permission for addition of 2'-FL to infant formula products (inclusive of GOS/ITF mixtures as per this Application) is unlikely to have a significant effect on other international trade as these substances are already permitted in similar products in countries overseas, including EU countries, the UK, USA, Israel and Asian countries.

## 4 Information to support the application

The application contains supporting information in accordance with the Application Handbook's requirements in Guideline 3.3.3 – Substances used for a nutritive purpose and Guideline 3.6.2 – Special purpose foods – Infant formula products. As noted in section 2, this application focusses on the safety and benefit of adding a combination of 2'-FL and GOS/ITF to infant formula products (sections B and E respectively).

The safety and benefit of the individual ingredients has previously been assessed by FSANZ. Chr. Hansen 2'-FL in A1190 and in the case of GOS/ITF, in P306. GOS/ITF has been approved for use in infant formula products for approximately thirteen years in Australia and New Zealand. Nutricia has been using a specific, patented combination of scGOS/lcFOS (9:1 ratio) in IFP for this time. During pre-application liaison, FSANZ requested that this application include consideration of relevant published literature relating to the safety and benefit of the individual ingredients since the respective approvals described above. A literature search was conducted to identify studies relating to 2'-FL in infant formula products in addition to studies identified in A1190. The results of this literature search are described in section C.5.1.

A further literature search was conducted to update information available to P306 in 2008 on the safety of GOS/ITF in infant formula products and any studies with GOS/ITF to infant formula products. The results of this literature search are described in section C.5.2.

## 5 Assessment procedure

*(addressing section 3.1.1.F of the FSANZ Application Handbook)*

The applicants consider the application should be assessed under the general procedure. FSANZ has previously conducted comprehensive safety and benefit assessments for the use of each ingredient in infant formula products. The use of both ingredients in combination does not require new assessments of each ingredient by FSANZ.

## 6 Confidential commercial information (CCI)

*(addressing section 3.1.1.G of the FSANZ Application Handbook)*

The application contains confidential commercial information (CCI) relating to the Nutricia infant formula manufacturing process. This information is provided in Appendix A. This information is of commercial value to Nutricia and has not been publicly released to date. Public release of this information can reasonably be expected to diminish the commercial value of this information to Nutricia.

## 7 Other confidential information

*(addressing section 3.1.1.H of the FSANZ Application Handbook)*

Confidential information relating to the research and manufacturing of the Chr. Hansen 2'-FL was contained in A1190 (Jennewein 2'-FL in Infant and Toddler Formulas). This information is not reproduced in this Application as it has not changed.

Nutricia and Chr. Hansen do not wish to identify any other part of this application as confidential information other then as stated in Part 6 above.

## 8 Exclusive capturable commercial benefit (ECCB)

*(addressing section 3.1.1.I of the FSANZ Application Handbook)*

Nutricia and Chr. Hansen are requesting exclusive permission, for a period of 15 months after gazettal of permission in the Code, for their respective brands to be added in combination to infant formula products. Nutricia and Chr. Hansen have invested significantly to develop their respective ingredients. This includes research and investment on ingredients and processes, development of patented technology, manufacturing capital expenditure and trials, sensory trials, shelf-life trails etc and conducting clinical trials (on the individual ingredients and a combination of both ingredients). The granting of an exclusive permission to Nutricia and Chr. Hansen will confer an exclusive capturable commercial benefit (ECCB) on the applicants. Exclusive permission will provide Nutricia and Chr. Hansen with opportunity to obtain a more significant return on the substantial research and investment costs that both companies have contributed to the development of infant formula products containing both ingredients.

Nutricia and Chr. Hansen consider it is possible that this application may also confer an exclusive capturable commercial benefit on the basis of the following points listed in guideline 3.1.1.H of the Application Handbook:

Handbook question: Why are you making this application? What are you hoping to get out its approval?

Response: The application seeks permission for the voluntary addition of the ingredients 2'-FL and GOS/ITF to infant formula products. This will allow further scientifically advanced, innovative, safe and high-quality infant formula products to be available for formula-fed infants. These products take advantage of scientific research to provide a formula that more closely resembles that of human milk. This is important to infants who, for whatever reason, are not breast fed as it provides them with excellent nutrition in early life. The benefits to consumers and industry are presented in Section 3 of the application. Infant formula products containing these ingredients are currently available in other countries, and permission to add these ingredients will enable products containing them to be sold in ANZ.

Handbook question: How will you benefit from the approval of your application?

Response: Approval of the application will enable infant formula products to be manufactured and sold in ANZ whilst also providing export access opportunities for both companies.

Handbook question: Who besides you, will benefit from the approval of your application? How and why will they benefit?

Response: Consumers will benefit from the approval of this application, as described above. Other manufacturers of infant formula products may benefit from being permitted to add a combination of 2'-FL and GOS/ITF to products that they manufacture.

Handbook question: If your application is approved, whose permission will be required before anyone can derive a benefit from that approval?
Response: Other parties wishing to use either the Chr. Hansen 2'-FL, or Nutricia's patented scGOS/LcFOS would need to enter into commercial agreements with the respective companies for access to and use of the ingredients in infant formula products.

Handbook question: Who holds the intellectual property in the subject matter of your application?
Response: Nutricia and Chr. Hansen hold the intellectual property for the patented mix ratio of scGOS/lcFOS (9:1 ratio) and the information relating to Chr. Hansen's 2'-FL.

# 9 International and other standards

*(addressing section 3.1.1.J of the FSANZ Application Handbook)*

## 9.1 International standards

Relevant Codex Alimentarius Commission Standards relevant to this Application include the *FAO/WHO Codex STAN 72-1981 Standard for Infant Formula and Formulas for Special Medical Purposes Intended for Infants. Revision 2007*. Of relevance to this Application are the following Sections from Codex STAN 72-1981:

- Section 3.2 (Optional Ingredients)
- Section 3.2.1 "In addition to the compositional requirements listed under 3.1.3, other ingredients may be added in order to provide substances ordinarily found in human milk and to ensure that the formulation is suitable as the sole source of nutrition for the infant or to provide other benefits that are similar to the outcomes of populations of breastfed babies."
- Section 3.2.2 "The suitability for the particular nutritional uses of infants and the safety of these substances shall be scientifically demonstrated. The formula shall contain sufficient amounts of these substances to achieve the intended effect, taking into account levels in human milk".

In addition, the *FAO/WHO Codex CXS 156-1987 Standard for Follow-Up Formula, Revision 2017* (FAO/WHO, 1987) is of relevance to this Application where it refers to formula for 6-12 month infants, in particular requirements of Section 3.3.2 (Optional ingredients):

- Section 3.3.2.1 "In addition to the vitamins and minerals listed under 3.2.4 to 3.2.6, other nutrients may be added when required to ensure that the product is suitable to form part of a mixed feeding scheme intended for use from the 6th month on."
- Section 3.3.2.2 "The usefulness of these nutrients shall be scientifically shown."
- Section 3.3.2.3 "When any of these nutrients is added, the food shall contain significant amounts of these nutrients, based on the requirements of infants from the 6th month on and young children".

## 9.2 Other national standards or regulations

### 9.2.1 Australia and New Zealand

There are no relevant compositional standards in Australia and New Zealand, other than the Code requirements for infant formula products identified in Standard 2.9.1 Infant formula products. The relevant compositional requirements for inulin-type fructans (ITF), galacto-oligosaccharides (GOS) and 2'-FL are reproduced below:

**2.9.1—7 Restriction on addition to infant formula product of inulin-type fructans and galacto-oligosaccharides**

(1) If an inulin-type fructan or a galacto-oligosaccharide is added to an infant formula product, the product must contain (taking into account both the naturally-occurring and added substances) no more than:
- (a) if only *inulin-type fructans are added—110 mg/100 kJ of inulin-type fructans; or
- (b) if only *galacto-oligosaccharides are added—290 mg/100 kJ of galacto oligosaccharides; or
- (c) if both inulin-type fructans and galacto-oligosaccharides are added:
  - (i) no more than 110 mg/100 kJ of inulin-type fructans; and
  - (ii) no more than 290 mg/100 kJ of combined inulin-type fructans and galacto-oligosaccharides.

(2) An infant formula product to which an inulin-type fructan or a galacto-oligosaccharide is added must not contain any of the following added substances:
- (a) 2′-O-fucosyllactose; or
- (b) a combination of 2′-O-fucosyllactose and lacto-N-neotetraose.

### 9.2.2 International

Food safety authorities in multiple jurisdictions have reviewed the safety of use of Chr. Hansen 2'-FL as an ingredient added to infant formula, follow-on formula, and toddler (young child) formula and the ingredient currently is marketed in these regions notably:
- the United States of America (multiple GRAS Notices, including GRN 571),
- the European Union (EFSA opinion 2015 and the European Commission Implementing Decision 2017),
- Canada (Health Canada 2020),
- Asia

Since September 2016, Chr. Hansen 2'-FL is found in multiple infant, follow-on and toddler (young child) formula products worldwide. In addition to the United States, the European Union, and Canada, Chr. Hansen 2'-FL currently is marketed in formulas for infants and toddlers worldwide including Argentina, Brazil, Mexico, Singapore, United Arab Emirates, and Israel, with permitted use levels ranging between 1.0 to 2.0 g 2'-FL per L infant formula as consumed. Other international permissions for use of 2'-FL include Korea and the Philippines, at maximum levels of 2 g/L and 1.2 g/L, respectively. In addition, 2'-FL is also authorized in Malaysia, Taiwan and Singapore.(A1190 pp16-18, 26-28, Jennewein 2’-FL in Infant and Toddler Formulas).

European Union
2'-FL, obtained by chemical synthesis, has been approved as a novel food ingredient for use in infant formula and follow-on formula at maximum level of 1.2 g/L in combination with 0.6 g/l of

lacto-N-neotetraose at a ratio of 2:1 (Commission Implementing Decision (EU) 2016/376 and Commission Implementing Decision (EU) 2016/375).

2'-FL produced with *Escherichia coli* strain BL21 has been approved as a novel food ingredient and is permitted to be added to infant formula and follow-on formula at maximum level of 1.2 g/l (Commission Implementing Decision (EU) 2017/2201). This includes the Chr. Hansen 2'-FL. Regulation (EU) 2017/2470 permits 2'-FL alone to be present at a maximum level of 1.2 g/L in infant formula and follow-on formula.

Inulin-type fructans and galacto-saccharides may be added to infant formula and follow-on formula (Commission Delegated Regulation (EU) 2016/127). In that case their content shall not exceed: 0.8 g/100 ml in a combination of 90 % oligo-galactosyl-lactose and 10 % high molecular weight oligo-fructosyl-saccharose. Other combinations and maximum levels of fructo-oligosaccharides and galacto-oligosaccharides may be used, provided that their suitability for infants is demonstrated in accordance with Article 3(3) (suitability of ingredients).

There is no restriction on the addition of both 2'-FL and GOS/ITF to the same infant formula or follow-on formula product provided that both substances comply with the requirements laid down in the applicable legislation. Nutricia has marketed its infant formula and follow-on formula product containing both 2'-FL and scGOS/lcFOS since 2018 in EU countries.

United States

Jennewein (Chr. Hansen) submitted a GRAS notification (GRN 571) to the United States (US) Food and Drug Administration (FDA) in 2015 for the use of 2'-FL in infant formula and toddler formulas at a maximum level of 2 g/L. This is the same 2'-FL ingredient assessed and approved by FSANZ in Application A1190. The FDA had no questions in relation to Chr. Hansen's conclusion that 2'-FL is GRAS under the intended conditions of use.

In the United States, 2'-FL, including Chr. Hansen 2'-FL, is GRAS for use in infant formulas at levels up to 2.4 g/L and selected conventional foods and beverages at levels ranging from 0.28 to 2.04 g/serving (GRN 546, 2015; GRN 571, 2015; GRN 650, 2016; GRN 735, 2018; GRN 749, 2018; GRN 852, 2019; GRN 897, 2020; GRN 929, 2021). Those listed GRAS Notifications filed with the USFDA received "no questions" letters.

Canada

Health Canada issued a Letter of No Objection in June 2020 to the use of 2'-FL produced from *E. coli* BL21 (DE3) strain as an ingredient in formula for term infants at levels up to 1.2 g/L of formula, as consumed.

Health Canada's review of the information presented in support of the use of 2'-FL derived from the production strain in infant formula did not raise concerns related to food safety (Health Canada 2020).

Brazil

The Brazilian Health Regulatory Agency, ANVISA has assessed and approved the addition of 2'-FL to infant formula and follow-on formula at a level of 1 g/L alone or in combination with 8 g/L of GOS and FOS (ratio 9:1). They concluded the consumption of infant formula and follow-on formula containing 2'-FL, GOS and FOS at these levels is safe for consumption by infants (ANVISA 2011a, 2011b, 2011c).

Singapore and Israel

The Singapore Food Agency (SFA) Sale of Food Act, Food Regulations CAP 283, Cl. 252, 6(g-j) permits the addition of 2'-FL up to 1.2 g/L and LNnT up to 0.6 g/L in infant formula products (Singapore Food Agency 2022). The regulation also permits a 2'-fucosyllactose / difucosyllactose (2'-FL /DFL) mixture that contains at least 75% (w/w) 2'-fucosyllactose and at least 5% (w/w) difucosyllactose for addition to infant formula (under Regulation 252(6)), in an amount not exceeding 1.6 g/L (in the case of infant formula for infants not more than six months of age) and 1.2 g/L (in the case of infant formula for infants more than six months of age but not more than twelve months of age).

The Israel Ministry of Health permits a maximum level of 2 g/L 2'-FL alone in infant formula products.

Taiwan

Taiwan approved the use of 2'-FL in nutrition products for infant and young children in 2021. According to the Taiwan Food and Drug Administration, 2'-FL can be used in infant formula, growing-up formula, and food or milk formula for children below seven years old.
The permitted amount for use is 1.2 g/L. (Taiwan Food and Drug Administration 2020).

## 10 Statutory Declaration

**STATUTORY DECLARATION**

Statutory Declarations Act 1959[1]

I, Rodrigo Lima, General Manager, Nutricia Australia Pty Ltd, Level 4 Building D, 12-24 Talavera Road, Macquarie Park NSW 2113 Australia

make the following declaration under the Statutory Declarations Act 1959:

1. the information provided in this application fully sets out the matters required
2. the information provided in this application is true to the best of my knowledge and belief
3. no information has been withheld that might prejudice this application, to the best of my knowledge and belief

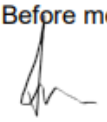
I understand that a person who intentionally makes a false statement in a statutory declaration is guilty of an offence under section 11 of the Statutory Declarations Act 1959, and I believe that the statements in this declaration are true in every particular.

Rodrigo Lima

Signature of Rodrigo Lima

Declared at Nutricia Australia Ltd, Level 4 Building D, 12-24 Talavera Road, Macquarie Park NSW 2113 on 05 of January 2022.

Before me,



Signature of Maria Venetoulis[2]

Maria Venetoulis, General Counsel, Nutricia Australia Pty Ltd, Level 4 Building D, 12-24 Talavera Road, Macquarie Park NSW 2113

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Rodrigo Lima (Jan 5, 2022 16:15 GMT+11)

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[1] http://www.comlaw.gov.au/Series/C1959A00052

[2] A statutory declaration must be made before a prescribed person under the Statutory Declarations Act 1959.
The list of prescribed persons is available in the Statutory Declarations Regulations 1993 at http://www.comlaw.gov.au/Series/F1996B00198

# A INFORMATION ON THE USE OF THE NUTRITIVE SUBSTANCE

*(addressing section 3.3.3.A of the FSANZ Application Handbook)*

## A.1 Information on the purpose of the use of a nutritive substance in food

*(addressing section 3.3.3.A.1 of the FSANZ Application Handbook)*

FSANZ has previously assessed the purpose of adding GOS/ITF and 2'-FL separately to infant formula products. Nutricia's GOS/ITF is added to IFP as a prebiotic that mimics the prebiotic effects and functional benefits of human milk prebiotics, providing a more favourable gut microbiota composition and activity than products that do not contain these ingredients. Chr. Hansen's 2'-FL is a HMO identical ingredient. 2'-FL is the most abundant HMO present in human milk and is added to IFP to provide a more compositionally similar profile to human milk and to provide bifidogenic effects and limit infection, including by pathogenic strains of *Campylobacter jejuni* in infants.

The combination of GOS/ITF and Chr. Hansen's 2'-FL is intended to be added to IFP to produce these independent effects but also to provide combined support to the immune system (through antipathogenic and direct effects), and bifidogenic effects.

More detail on the individual and combined purpose of addition of GOS/ITF and 2'-FL is described below.

<u>Purpose of GOS/ITF</u>

Human milk is the best source of nutrition for infants as it contains the right balance of nutrients and bioactive compounds to ensure optimal growth and development of the body and its organs, including the immune system, the gut and gut microbiota and the brain. Human milk contains many bioactive compounds such as oligosaccharides, immune cells and bacteria and their metabolites, which play a role in the development of a healthy immune system by supporting the development of a balanced gut microbiota and providing anti-infective and immune development stimulating properties (Bergmann et al. 2014; Brand-Miller et al. 1998; Engfer et al. 2000; Fernández et al. 2013).

In human milk, the proportion of prebiotic carbohydrates is substantial (12-15 g/L), whereas prebiotic oligosaccharides in cow's milk are present only in trace amounts. Currently, approximately 200 oligosaccharide structures in human milk have been elucidated, and many more are present, at least in small quantities (Thurl et al. 2017).

Oligosaccharide structures that display prebiotic effects similar to HMOs include short chain galacto-oligosaccharides (GOS) and ITF (including long chain fructo-oligosaccharides (lcFOS)), globally the most studied prebiotic mixture. The mix is in the range of HMOs close to human milk in quantity and diversity. Although this prebiotic mixture approaches the molecular size distribution of short and long-chain oligosaccharides in human milk, it is not structurally similar to HMOs (Salminen et al. 2020). The mixture has been shown to beneficially modulate the gut microbiota, including an increase in the faecal level of bifidobacteria, to result in a gut ecosystem similar to breast-fed infants. The mixture can also positively affect stool characteristics, bringing them closer to breast-fed infants (Knol et al. 2005; Moro et al. 2002; Salvini et al. 2011, Scholtens et al. 2008). Moreover, in clinical studies (Scholtens et al. 2014 and Vandenplas et al. 2017) a stool softening effect has been reported as well as to reduce

infections in formula-fed heathy infants (Arslanoglu et al. 2007, Arslanoglu et al. 2008, Boehm et al. 2007 and Salminen et al. 2020).

Therefore, the specific mixture of prebiotic oligosaccharides (such as scGOS/lcFOS) exerts a beneficial nutritional and physiological function in infants and toddlers (or in early life). The level of 0.8 g/100 ml GOS/ITF that is currently permitted in infant formula in Standard 2.9.1 has been shown to induce a physiological benefit with respect to the composition of the intestinal microbiota, and its metabolic activity.

Clinical studies reported a stool softening effect through addition of a specific mixture of scGOS/lcFOS in infant formula (Scholtens et al. 2014 and Vandenplas et al. 2017). This specific mixture of scGOS:lcFOS has also been shown to reduce infections in formula-fed heathy infants (Arslanoglu et al. 2007 and Arslanoglu et al. 2008).

In a review of current research (range 2005-2019), Yang et al. (2020) reported that there was consistent clinical evidence that dietary fibres, including arabinoxylans, galacto-oligosaccharides, inulin, and oligofructose, promote a range of beneficial bacteria and suppress potentially detrimental species in the gut. Abrahamse-Berkeveld et al. (2016) reported that infant formula containing GOS/ITF and *Bifidobacterium breve* M-16V supported adequate growth and tolerance in healthy infant clinical trials.

Purpose of 2'-FL

A1155 (A1155 Application final) and A1190 (Jennewein 2'-FL in Infant and Toddler Formulas) provided the detailed purpose for adding 2'-FL to infant formula. As the most prevalent of the HMOs found in human breast milk, it is reported to have a role in the gut and immune system of infants (Lewis et al. 2015, Morrow et al. 2004 and Siziba et al. 2021), reduce risk for lower respiratory tract illnesses through a protective effect on mucosal barrier function (Sprenger et al. 2019) and an immunomodulation role in prevention of allergic diseases in early life (Zuurveld et al. 2020).

Purpose of combination of 2'-FL and GOS/ITF

The addition of both 2'-FL and GOS/ITF to infant formula and follow-on formula at the intended levels (up to 2.4 g/L and 8 g/L respectively) is consistent with average levels of HMO present in human milk and is intended to provide formula-fed infants with oligosaccharide intake levels closer to those of breastfed infants that contain either ingredient individually. The addition of both 2'-FL and GOS/ITF to infant formula products is expected to provide infants with the benefits associated with 2'-FL while also providing the benefits associated with GOS/ITF.

A study by Vandenplas et al. (2020), which was a randomised, double-blind, controlled design study, using a specific GOS:FOS mixture (9:1 ratio) and 2'-FL, demonstrated support for adequate infant growth, whilst being safe and well-tolerated in healthy-term infants. Evidence from this study and other clinical studies on IFP containing added 2'-Fl or both 2'-FL and GOS show support for the immune system through antipathogenic and direct effects, as measured by less infections (Puccio et al. 2017) and a more favourable inflammatory cytokine profile (Goehring et al. 2016). Berger et al. (2020) demonstrated a favourable benefit on the gut (bifidogenic effect) when feeding a formula with 2 HMOs (2'-FL and LNnt) in comparison to breastfed reference infants' microbiota.

There are a number of other studies where the beneficial effect of GOS/ITF and 2'-FL on gut microbiota, bifidogenic effects and stool consistency characteristics in infant formula have been reported (Huet et al. 2016, Neumer et al. 2021, Rodriguez-Herrera et al. 2019 & Vandenplas et al. 2015). See Section E for more detail.

## A.2 General data requirements for supporting evidence

*(addressing section 3.3.3.A.2 of the FSANZ Application Handbook)*

The studies referenced in this application in support of safety and the purpose of addition have been conducted on the commercial preparation produced by Nutricia. Studies on human subjects consuming infant formula products are directly relevant to the intended addition of the GOS/ITF and 2'-FL combination to infant formula products proposed in this application. The description of the studies in the application includes the experimental methods used and copies of all studies have been provided to FSANZ.

# B TECHNICAL INFORMATION ON THE USE OF THE NUTRITIVE SUBSTANCE

Typically, applications relating to permissions for substances used for a nutritive purpose must provide information to demonstrate the properties of the substance are suitable for the intended incorporation into food products. FSANZ has already assessed this information for the individual ingredient permissions given in Standard 2.9.1 for the 2'-FL and GOS/ITF ingredients that form this Application.

## B.1 Information to enable identification of the nutritive substance

Application A1190 (Jennewein 2'-FL in Infant and Toddler Formulas) and Proposal P306 (FOS & GOS Final) provide detailed information on the identity of 2'-FL and GOS/ITF respectively, for which permission is sought in this Application.

## B.2 Information on the chemical and physical properties of the nutritive substance

The chemical and physical properties of 2'-FL have been extensively assessed by FSANZ. A1190 (Jennewein 2'-FL in Infant and Toddler Formulas p41-51) includes information on the chemical and physical properties of the proposed 2'-FL. GOS and ITF chemical and physical properties were addressed in detail in FSANZ's Proposal P306 (FOS & GOS Final, Attachment 2 p26-27). Combining these 2'-FL and GOS/ITF ingredients in infant formula products will not change the chemical and physical properties of the respective ingredients.

Shelf-life studies conducted by Danone Nutricia Research B.V. (Nutricia Research 2021) confirm that the A1190 2'-FL, at levels ranging between 2.9 - 4.0 g/L, added to infant formula containing 8 g/L scGOS & lcFOS, is stable in IFP for at least 18 months at 20°C and 30°C.

## B.3 Information on the impurity profile

A1190 (Jennewein 2'-FL in Infant and Toddler Formulas p92-104) included detailed information on controls put in place during the production of the Chr. Hansen 2'-FL product to ensure the genetically modified source organism (*Escherichia coli*) is not present in the final 2'-FL product that is incorporated into infant formula products. Nutricia also has strict quality control processes in place during the manufacture of its GOS/ITF mixture to ensure its purity profile as provided in Proposal P306 (FOS & GOS Final) and Application A1055 (Application) .

## B.4 Manufacturing process

1. The manufacturing process for the Chr. Hansen 2'-FL ingredient is described in Application A1190 (Appendix K) and is not reproduced in this application.

2. Nutricia's GOS/ITF mixture is manufactured in summary by:

- Short chain galacto-oligosaccharides (scGOS):
  - the raw material is syrup, which is produced from lactose by enzymatic conversion into galacto-oligosaccharides;
  - scGOS is a soluble fibre with a chemical composition of glucose (galactose)n in which "n" varies up to 7.

- Long chain Fructo-oligosaccharides (lcFOS):
  - the raw material is a powder, obtained after fractionation of inulin;
- lcFOS is a soluble fibre;
- the official name is “high molecular weight oligofructosyl-saccharose”.
- LcFOS is a soluble fibre with a chemical composition of glucose(fructose)n in which ‘n’ varies between 7 and 60; the average is 23.

scGOS and lcFOS are supplied to Danone Nutricia B.V. by several suppliers.

The manufacturing process for infant formula and follow-on formula containing both 2'-FL and GOS/ITF is described in Appendix A (confidential).

In summary, the infant formula powder manufacturing process consists of three stages:

1. Production of a spray dried base powder, from pasteurised cow’s milk and other ingredients, followed by:
2. Dry blending of base powder and other added ingredients and
3. Final packaging

The manufacturing processes of the wet blending and dry blending phases are conducted in manufacturing facilities that are registered and approved as dairy manufacturing premises, HACCP certified and subject to official, systematic government audit. The manufacturing facilities are ISO and accredited food safety systems certified.

### B.5 Specification for identity and purity

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. Nutricia’s GOS/ITF mixture has been used as an ingredient in infant formula products in Australia and New Zealand for approximately thirteen years. A specification for GOS/ITF is not included in Schedule 3 of the Code. The existing Code requirements for identity and purity do not require amendment for the intended use of these ingredients that are already separately permitted to be added to infant formula products.

### B.6 Analytical method for detection

Analytical methods for detection are available in detail in previously considered application and proposal for both 2'-FL (A1190 - (Jennewein 2’-FL in Infant and Toddler Formulas Appendix N) and GOS/ITF (P306 – (FOS & GOS Final).

### B.7 Information on the proposed food label

This requirement is addressed in section F in accordance with specific Application Handbook labelling requirements for special purpose foods.

## C Information related to the safety of the nutritive substance

This section addresses the information requirements in the FSANZ Application Handbook for Guidelines 3.3.3 (Nutritive Substances) and 3.6.2 (Special Purpose Foods – Infant Formula Products). The following table sets out which parts of this section address each of the Guideline requirements. The terminology for GOS/ITF was developed by FSANZ in 2008 to ensure clarity about a range of terms that had been used for describing galacto-oligosaccharides, inulin-derived substances and fructo-oligosaccharides. The term GOS/ITF is used throughout this application for consistency with FSANZ terminology. Pre-application discussions with FSANZ identified that the oligosaccharide load of infant formula containing both 2'-FL and GOS/ITF should be discussed in the context of the safety of consumption for infants and comparison with the oligosaccharide load of human milk. This issue is discussed in section C.1. Other aspects relating to the safety of a combination of 2'-FL and GOS/ITF in infant formula products is discussed in sections C.2 to C.4.

As noted in section 4 above, the application also includes consideration of safety data relating to the individual ingredients that has become available after FSANZ's respective approvals of 2'-FL and GOS/ITF. The results of literature searches conducted for both 2'-FL and GOS/ITF are described in section C.5.

| Handbook Guideline | Requirement | Application section |
|---|---|---|
| 3.3.3 – Nutritive substances | C.1 Information on the toxicokinetics and metabolism of the nutritive substance | C.2 |
| | C.2 Information from studies in animals or humans relevant to toxicity | C.3, C5 |
| | C.3 Safety assessment reports prepared by international or national government agencies | C.4 |
| 3.6.2 – Special purpose foods – Infant formula products | A.3.1.b Nutritional safety and tolerance of the proposed compositional change | C.3 |

### C.1 Oligosaccharide content of human milk and infant formula

Pre-application discussions with FSANZ identified that the oligosaccharide load of infant formula containing 2'-FL at up to 2.4 g/L and GOS/ITF at up to 8 g/L (at a 9:1 ratio of GOS:FOS) should be considered in the context of the oligosaccharide load of human milk. The intended use of 2'-FL and GOS/ITF in infant formula and follow-on formula will therefore result in a total 2'-FL and GOS/ITF content of 10.4 g/L of these oligosaccharides. This is less than the reported average oligosaccharide load of human milk.

The oligosaccharide content of human milk was investigated in a systematic review by Thurl et al. (2017). They noted that HMO content can vary across individuals, by gestational age and by the lactation period. However, they identified twenty-one studies that reported on HMO content and determined average quantities of HMO reported in studies of term and pre-term milks. The average quantity of neutral HMOs present in term milk of secretor mothers was 14.78 g/L, which was higher than pre-term milk (average = 11.57 g/L). This compares favourably to the levels of HMO in mature human milk of 10-15 g/L reported by Jantscher-Krenn et al. (2012). The 10-15 g/L total HMO range is also consistent in an earlier publication of Kunz and Rudloff (2006).

Health Canada (2020) in its acceptance of addition of the Chr. Hansen (formerly Jennewein) 2'-FL concluded that "as bovine milk contains less oligosaccharides than human milk, and specifically less 2'-FL, infants on un-supplemented bovine milk formulas are currently exposed to less oligosaccharides and 2'-FL than human milk fed infants. The dietary exposure for infants consuming 2'-FL in infant formula would be lower or consistent with current exposure for breastfed infants, as the proposed rate of addition of 2'-FL in formulas is lower than that in breast milk. Breast milk contains on average 2.38 g of 2'-FL /L (Erney et al. 2000), which is about two-fold higher than the petitioner's proposed maximum specification of 1.2 g of 2'-FL /L (includes overage)". In A1155 and A1190, a level of 2.4 g/L 2'-FL was approved for addition to IFP.

## C.2 Information on the toxicokinetics and metabolism of the nutritive substance

The safety of Chr. Hansen's 2'-FL and GOS/ITF have been assessed separately by FSANZ, in A1190 (Approval Report, p 20-21) and P306 ( FOS & GOS Final) respectively. Nutricia submitted A609 (GOS, Long Chain Inulin Final) in 2007 seeking permission to added GOS and long chain inulin (ratio 9:1) at a maximum level of 8 g/L respectively. FSANZ dealt with the aspects of A609 concurrently with its assessment of P306.

In summary, in both of these assessments FSANZ concluded there were no safety concerns associated with the addition of:

- 2'-FL at levels of 2.4 g/L permitted in the Code from another derivation of 2'-FL, or
- GOS/ITF, alone or in combination, to IFP to a total maximum of 290 mg/100 kJ (0.8 g/100 mL)

The focus of this application is demonstrating the safety of IFP containing a combination of 2'-FL and GOS/ITF. Section C.3 expands upon the safety, based on results of a clinical study in infants and additional relevant studies.

## C.3 Information from studies in animals or humans relevant to toxicity

In this section, the results of the Danone Nutricia clinical trial with 2'-FL, as reported by Vandenplas et al. 2020 are presented (section C.3.1). In addition, a PubMed search was performed to identify relevant studies. A summary of the relevant identified studies is included below in section C.3.2.

### C.3.1 Vandenplas et al. (2020)

The results of this clinical study provide evidence for the safety of an infant formula (IF) (Lactofidus™ 26% fermented IF) with 2'-FL, scGOS/lcFOS (9:1) also containing postbiotics derived from the Lactofidus™ fermentation process (including 3'-Galactosyllactose (3'-GL)).

Study design
In this double-blind, randomized, controlled, parallel group, multi-country study trial, healthy fully IF-fed infants were randomised ≤14 days of age to one of two formulas until 17 weeks of age:

1. A 26% LactofidusTM fermented IF with scGOS/lcFOS (9:1), 2'-FL and milk fat (test group) or
2. an IF with scGOS/lcFOS (9:1) (control group).

A group of exclusively breastfed (BF) infants served as reference.

*Study objectives*
This study investigated growth, safety and tolerance of the intervention formula. The primary objective of the study was to demonstrate the equivalence of daily weight gain during the intervention period between IF groups. The secondary objectives of this study were:

- To test equivalence of weight gain per day from baseline until the age of 17 weeks in subjects receiving the test product compared to subjects receiving human milk.
- To test equivalence of other growth parameters from baseline until the age of 17 weeks in subjects receiving the test product compared to subjects receiving the control product or subjects receiving human milk.
- To evaluate z-scores development for growth parameters from baseline until the age of 17 weeks in subjects receiving the test product compared to subjects receiving the control product or subjects receiving human milk.
- To assess GI tolerance from baseline until the age of 17 weeks in subjects receiving the test product compared to subjects receiving the control product.
- To assess the safety of the test product compared to the control product from baseline until the age of 17 weeks.

The exploratory objectives of this study were:

- To investigate faecal microbiota composition and functionality from baseline until the age of 17 weeks in subjects receiving the test product compared to subjects receiving the control product or subjects receiving human milk.
- To investigate the incidence of infection and concomitant medication usage from baseline until the age of 17 weeks in subjects receiving the test product compared to subjects receiving the control product or subjects receiving human milk.
- To investigate immune parameters in stool, saliva and blood samples from baseline until the age of 17 weeks in subjects receiving the test product compared to subjects receiving the control product or subjects receiving human milk.

*Study population*
Included were 215 healthy fully IF-fed infants (108 Test group, 107 Control group) randomised ≤14 days of age and a group of 61 exclusively breastfed (BF) infants.

*Anthropometric results*
As the primary outcome, equivalence in daily weight gain (g/d) between the test group and the control group from baseline to 17 weeks of age was demonstrated. The difference in the estimated means (SE) of daily weight gain between the two groups was -0.08 (0.84) g/d, 90% CI -1.47, 1.31. The estimated mean (SE) daily weight gain was 31.0 (0.59) g/d in the test group and 31.08 (0.60) g/d in the control group. Equivalence was also confirmed for both length and head circumference gain between the test and control groups.

The mean (SE) daily weight gain of the breastfed reference group of 28.3 (0.79) g/d appeared slightly lower than the test group and was not equivalent with an estimated mean (SE) difference of 2.65 (0.99) g/d, 90% CI (1.01, 4.29). Equivalence was confirmed for both length and head circumference gain between the test group and breastfed reference group.

In comparison with the WHO growth standards, the estimated z-scores for weight-for-age, length-for-age, BMI-for-age, and head circumference-for-age of the randomised groups as well as the breastfed reference group were all within +/- 1 SD bandwidth, indicative for adequate infant growth.

*Formula intake*
During the intervention period, completion of the feeding diaries prior to the visits ranged between 90-98% of all randomised infants enrolled in the study. No statistically significant differences in IF intake were observed between the test and control groups at any timepoint. Similarly, there were no statistically significant differences in intake per kg body weight (mL/kg/d) between the groups.

*Results adverse events*
At least one adverse event (AE) was reported in 39.3% of infants in the test group, 31.7% in the control group with an estimated risk difference of 7.52% and a corresponding 95% CI of -5.42%, 20.22% (P = 0.255). For reference, an AE was reported for 24.6% of infants in the breastfed reference group.

The most common AEs occurred in the system organ class of gastrointestinal disorders which were reported in 20.6% of infants in the test group, 16.3% in the control group, and 9.8% in the breastfed reference group. Infections and infestations were reported in 15.9% of infants in the test group, 16.3% in the control group, and 13.1% in the breastfed reference group.

In contrast to the parent-reported stool characteristics where no infrequent hard stools were noted in the randomised groups, investigators reported constipation for 4 (3.7%) infants in the test group, 3 (2.9%) infants in the control group, and 1 (1.6%) infant in the breastfed reference group. There were no statistically significant differences between the randomised groups in the number of total or specific AEs.

A total of 11 serious AEs (SAEs) were reported in the randomised infants with 7 events reported in 6 (5.6%) infants in the test group and 4 events in 4 (3.8%) infants in the control group (P>0.05). From these SAEs 6 events in 5 (4.7%) infants in the test group and 3 events in 3 (2.9%) infants in the control group were in the System Organ Class of infection and infestation. The remaining two events were a case of infantile vomiting in the control group and a case of rhesus incompatibility in the test group. No statistically significant differences in any SAEs were observed between the test and control groups. All serious adverse events were described by the investigator as not related or unlikely related to the study product. No SAEs were reported for the breastfed reference group.

*Gastrointestinal tolerance*
No statistically significant differences were observed in gastrointestinal tolerance and stool consistency between randomised IF groups.

*Conclusion*
No safety signals were observed with the formulation studied. No statistically significant differences were observed in adverse events or gastrointestinal tolerance between randomised IF groups.

C.3.2 Supportive studies

A PubMed and manual search on human clinical trials including the search terms 2'-FL, 2'-FL, 2'fucosyllactose or 2'-Fucosyllactose was performed, resulting in 7 relevant publications. One was excluded from this section as it was an intervention in adults (Elison et al. 2016) and one was excluded as the study topic was fucosyltransferase 2 and human milk 2'-FL in relation to allergy (Sprenger et al. 2017). Of the remaining five publications, four included data on nutritional safety and suitability for infants and are summarised below (Marriage et al. 2015, Kajzer et al. 2016, Puccio et al. 2017, Storm et al. 2019). The HMOs 2'-FL and LNnt in infant formula have been the topic of several reviews (Vandenplas et al. 2018, Salminen et al. 2020, Vandenplas et al. 2020) and a narrative review of clinical trials with 2'-FL was written by Reverri et al. (2018). In addition, a real-world evidence study with an infant formula with added 2'-FL was found (Román Riechmann et al. 2020).

The four studies reporting data on nutritional safety and suitability for infants are discussed below.

Marriage et al. (2015) examined the growth and tolerance of infants fed IFs which had a caloric density closer to human milk and were supplemented with HMOs (2'-FL and GOS). They performed a prospective, randomised, controlled, growth and tolerance study in healthy, singleton infants (birth weight ≥ 2490 g), who were enrolled by day of life (DOL) 5. Fully formula-fed infants were randomized to 1 of 3 formulas with a caloric density of 64.3 kcal/dL; control formula (CF), or two experimental formulas (EFs) that were similar to the CF, except they contained GOS and varying levels (0.2 or 1.0 g/L) of 2'-FL. A group of breastfed infants served as reference. Infants were exclusively fed either formula (n=189) or human milk (HM; n=65) from enrolment to DOL 119. 2'-FL was measured in the blood and urine collected from a subset of infants at DOL 42 and 119, and in HM collected at DOL 42.

The results showed no significant differences between any of the intervention groups for weight, length, or head circumference gain during the 4-month study period, nor compared to the breastfed reference group. All formulas were well tolerated and no apparent differences in average stool consistency, number of stools per day, and percent of feedings associated with spitting up or vomiting existed. Overall, there were no safety concerns noted with either of the EFs. 2'-FL was present in the plasma and urine of formula-fed infants fed 2'-FL, relative absorption and excretion of 2'-FL were similar to those of breastfed infants. The authors concluded that infants fed 2'-FL fortified formulas with a caloric density similar to human milk show a similar growth and 2'-FL uptake to human milk fed infants. In addition, the formula was well-tolerated.

Kajzer et al. (2016) evaluated gastrointestinal tolerance of infants fed IF supplemented with scFOS and 2'-FL. They conducted a prospective, randomized, multi-centre, double-blinded, controlled 3-arm tolerance study in full term, singleton infants (birth weight ≥ 2490g) enrolled between 0 and 8 days of age. At enrolment, formula-fed infants were randomised to one of two experimental milk-based IFs with a caloric density of 64.3 kcal/dL. Experimental Formula 1 (EF1) did not contain oligosaccharides (n=42) and Experimental Formula 2 (EF2) contained 2 g/L scFOS and 0.2 g/L 2'-FL (n=46). The two formula groups were compared with a human milk-fed (HM) reference group (n=43). Infants were exclusively fed formula or human milk from enrolment until 35 days of age. Data related to intake, stool patterns, anthropometrics and parental questionnaires were collected. The primary outcome was average mean rank stool consistency (MRSC) from Study Day 1 to Visit 3. MRSC was calculated from stool records (1=watery, 2=loose/mushy, 3=soft, 4=formed, 5=hard).

Thirty-six (86%) subjects in the EF1 group, 41 (89%) in the EF2 group and 42 (98%) in the HM group completed the study. There were no differences among groups for gender, ethnicity, race, gestational age, birth weight or age at enrolment. From Study Day 1 to Visit 3, there were no differences in MRSC and stool consistency among the three feeding groups. The average number of stools per day for the HM group was significantly greater than EF1 ($p<0.0001$) and EF2 ($p<0.0001$) from Study Day 1 to Visit 3. At Visit 3, there were no differences between groups for average volume of study formula intake, number of study formula feedings per day, anthropometric data or percent feedings with spit-up/vomit.

The authors concluded that the formula with 2'-FL and scFOS was safe and well tolerated in infants, as evidenced by stool consistency, formula intake, percent feedings with spit-up/vomit, and reported AEs like those of the infants who were fed formula without oligosaccharides or those of the BF infants. This study was initially published as an abstract, but more details are described in the review by Reverri et al. (2018).

Puccio et al. (2017) evaluated the effects of IF supplemented with two HMOs on infant growth, tolerance, and morbidity. Healthy infants, 0 to 14 days old, were randomised to an intact protein, cow's milk–based IF (control, n=87) or the same formula with 1.0 g/L 2'-FL and 0.5 g/L lacto-N-neotetraose (LNnt) (test, n=88) from enrolment to six months. All infants received standard follow-on formula without HMOs from six to twelve months. The primary endpoint was weight gain through four months, secondary endpoints included additional anthropometric measures, gastrointestinal tolerance, behavioural patterns, and morbidity through the age of twelve months.

The results showed that weight gain was similar in both groups (mean difference [95% CI test vs control: -0.30 [-1.94, 1.34] g/day; lower bound of 95% confidence interval was above non-inferiority margin [-3 g/day]). No differences in other growth outcomes were observed between groups. Moreover, growth of both groups tracked closely with the WHO growth standards. Incidence of gastrointestinal symptoms and behavioural patterns were similar between groups, with exceptions including softer stools (P=0.021) and fewer night-time wakeups (P=0.036) in the test group at two months. No differences were observed in number, type or severity of adverse events between groups. The authors conclude that IF with 2'-FL and LNnt is safe, well-tolerated, and supports normal, age-appropriate growth.

Storm et al. (2019).evaluated the feeding tolerance of 2'-FL in a 100% whey, partially hydrolysed IF with the probiotic *Bifidobacterium animalis ssp. lactis* strain Bb12 (B lactis; test) as compared with the same formula without 2′FL (control) in a randomized controlled trial of healthy infants enrolled at 2 weeks of age (±5 days). After 6 weeks of feeding the assigned formula, the primary outcome of tolerance was assessed using the Infant Gastrointestinal Symptom Questionnaire. Stooling, vomiting, spit-up, crying, and fussing were compared between groups. Seventy-nine infants were enrolled and 63 completed the study per protocol (30 Test, 33 Control). Infant Gastrointestinal Symptom Questionnaire scores were similar between groups (test 20.9 ± 4.8, Control 20.7 ± 4.3, P = .82). It was concluded that partially hydrolysed IF with 2′FL and B lactis is tolerated well, as confirmed by a validated multi-symptom index.

C.3.3 *Summary and conclusion*

The Vandenplas clinical study (Vandenplas et al. 2020) concluded that the infant formula product studied containing 8 g/L scGOS/lcFOS (9:1) and 1 g/L 2'-FL, is safe and suitable for infants.

This conclusion is supported by the results of other clinical trials in which the addition of 2'-FL to IF (with intact or partly hydrolysed protein) with or without other oligosaccharides such as GOS (Marriage et al. 2015), scFOS (Kajzer et al. 2016) or LNnt (Puccio et al. 2017) or probiotics (Storm et al. 2019) has been shown safe and suitable for infants. This is in line with the conclusions of several reviews (Vandenplas et al. 2018, Salminen et al. 2020, Vandenplas et al. 2020) and an observational real-world evidence study (Román Riechmann et al. 2020).

Table 1: Summary of clinical trials evaluating safety and suitability of 2'-FL

| | **Vandenplas et al. 2020** | **Marriage et al. 2015** | **Kajzer et al. 2016** | **Puccio et al. 2017** | **Storm et al. 2019** |
|---|---|---|---|---|---|
| **Study product** | 26% fermented IF with 8 g/L scGOS/lcFOS (9:1), 1 g/L 2'-FL, fermentation metabolites and milk fat | IF with a lower caloric density and 2.2 g/L GOS, with or without<br>2 levels of 2'-FL: 0.2 and 1.0 g/L | IF with a lower caloric density with or without HMOs:<br>EF1: no HMOs<br>EF2: 2 g/L scFOS and 0.2 g/L 2′FL | IF with or without 1.0 g/L 2'-FL and 0.5 g/L LNnt. | 100% whey, partially hydrolysed IF with *Bifidobacterium animalis ssp. lactis,* with or without 0.25 g/L 2'-FL |
| **Safety results** | Equivalence in weight, length and head circumference gain. No significant differences in adverse events or gastrointestinal tolerance. | No significant differences among any groups for weight,<br>length or head circumference growth during the 4-month study period | The experimental formula containing 2′FL and scFOS was well tolerated, similar to that of infants fed formula without oligosaccharides or HM. | Weight gain was similar in both groups | Infant Gastrointestinal Symptom Questionnaire scores were similar between groups |

There is some evidence that consumption of prebiotics may prevent acute infections in young children (Lohner et al. 2014; Luoto et al. 2014)

Other animal and in vitro studies have indicated that prebiotics can stimulate the growth of bifidobacterial in the gut and provide protection against infection from pathogenic bacteria, by preventing their adhesion to the gut mucosa and invasion into tissues (Searle et al. 2009; Gouveia et al. 2013).

In a study on neonatal piglets, Comstock et al. (2017) reported that dietary HMOs were effective in altering systematic and gastrointestinal immune cells, reducing the duration of diarrhea after rotovirus infection. Azagra-Boronat et al (2018) reported that supplementation with 2'-FL and scGOS/lcFOS ameliorated rotavirus-induced diarrhea in suckling rats.

Szklany et al. (2020) investigated the effects of dietary supplementation with a specific mixture of GOS/ITF fed from the day of birth onwards in mice on behaviour and intestinal microbiota development. Behavioural tests were performed pre-weaning, in adolescence, early adulthood and adulthood. The authors assessed faecal microbiota compositions over time, caecal short-

chain fatty acids as well as brain mRNA expression of Htr1a, Htr1b and Tph2 and monoamine levels. When compared to control fed mice, the GOS/ITF mixture fed mice showed reduced anxiety-like and repetitive behaviour over time and improved social behaviour in adulthood.

### C.4 Safety assessment reports prepared by international or national government agencies

FSANZ has assessed and approved the use of two specific 2'-FL at up to 2.4 g/L in infant formula and follow-on formula (Applications A1150 & A1190). FSANZ has also previously assessed and approved the use of GOS/ITF at 8 g/L.

2'-FL has been assessed and approved as a novel food ingredient in the EU. The EFSA Panel on Diabetic Products, Nutrition and Allergies (NDA) provided a scientific opinion at the request of the European Commission in 2015 (EFSA 2015). The Panel concluded the addition of 2'-FL to infant and follow-on formulae at up to 1.2 g/L is safe. The Panel's opinion was based on the observations from a sub-chronic 90-day toxicity study in rats, where the no observed adverse effect level was 2 000 mg/kg body weight per day. A double-blind, randomised, controlled clinical trial on the effects of 2'-FL consumed in combination with another oligosaccharide (lacto-N-neotetraose (LNnt)) in infants was submitted and the Panel concluded that 2'-FL was safe for infants (up to one year of age) when added to infant formula and follow-on formulae, in combination with LNnt, at concentrations up to 1.2 g/L of 2'-FL and up to 0.6 g/L of LNnt, at a ratio of 2:1 in the reconstituted formulae.

The Brazilian Health Regulatory Agency (ANVISA) assessed and approved the addition of 2'-FL to infant formula and follow-on formula at a level of 1 g/L alone or in combination with 8 g/L of GOS and FOS (ratio 9:1). They concluded the consumption of infant formula and follow-on formula containing 2'-FL, scGOS and lcFOS at these levels is safe for consumption by infants (ANVISA 2011a, 2011b, 2011c).

Health Canada (2020) assessed that the addition of 2'-FL as a novel food ingredient, did not raise concerns related to food safety, including chemical, microbial, toxicological and allergenicity safety. Health Canada issued a Letter of No Objection to the use of 2'-FL produced from *E. coli* BL21 (DE3) strain as an ingredient in formula for term infants at levels up to 1.2 g/L of formula, as consumed. Health Canada's review of the information presented in support of the use of 2'-FL derived from the production strain in infant formula did not raise concerns related to food safety.

The Food Safety Authority of Ireland (FSAI) published a substantial equivalence opinion in 2016 (FSAI 2016) allowing the use of a 2'-FL produced by fermentation.

### C.5 Updated safety consideration of individual 2'-FL and GOS/ITF ingredients

#### C.5.1 2'-FL

In addition to the safety and benefit literature search conducted in the A1190 application, three further studies have been identified as further support for safety and benefit of 2'-FL added to IFP. Hegar et al. (2019) concluded that addition of 2'-FL alone was a safe supplementation for infant formula. The authors stated that the gastrointestinal microbiome of infants fed with a formula supplemented with 2′-FL was similar to that of infants in their study who were exclusively breastfed. They reported no adverse effects reported to date for 2'-FL with clinical studies demonstrating that infants fed on a formula supplemented with 2'-FL exhibit a normal

growth pattern and normal defecation. In a randomised multicentre trial, Puccio et al. (2017) fed infants with a formula supplemented with 2'-FL or LNnt from less than 14 days of life to the age of 6 months (4 months exclusively). The formula was reported to be safe and well-tolerated and supported an age-appropriate growth. Additionally, there was no difference in the stool consistency and stool frequency between the 2'-FL-fed group and the LNnt-fed group, except at 2 months when stools were softer in the 2'-FL-fed group. Fonvig et al. (2021) concluded from a randomised, double-blinded, placebo-controlled trial of 75 overweight children ages 6-12 years that 2'-FL or a mix of 2'-FL and LNnt was safe and well tolerated over an eight-week period, and beneficially modulated intestinal microbiota by increasing the numbers of bifidobacteria in the gut.

For this Application, a literature search was undertaken to provide further support for the safety of addition of either GOS/ITF in infant formula products or in combination (GOS/ITF and 2'-FL) to supplement previous application approvals.

The scoping review for the search was planned and conducted using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines extension for scoping reviews (PRIMSA-ScR) (Moher et al. 2015).

The aim was to address the following question: “What is known about the safety of adding 2'-FL to infant formula products already containing galactooligosaccharides (GOS) and fructooligosaccharides (FOS) or inulin-type fructans (ITF)?”

For the search strategy and eligibility criteria, a series of comprehensive systematic electronic literature searches was performed to find relevant studies reporting on the safety of adding 2'-FL to infant formula products already containing galactooligosaccharides (GOS) and fructooligosaccharides (FOS) or inulin-type fructans (ITF).

Recommended health and nutrition electronic databases were searched on 24 November 2021 at 13:00 AEST. These databases included: Scopus, Web of Science, Global Health (CABI) and Medline (incl. PubMed). The following search strategy was applied to terms listed within the titles, abstracts and keywords of articles: “infant formula AND galactooligosaccharides AND fructooligosaccharides OR inulin-type fructans AND 2'-FL”.

The review includes original research papers that reported on investigations conducted on human subjects, published as full-length articles in English. It excludes conference abstracts, editorials, letters to the editor and case reports. It includes randomised controlled trials, observational cohort studies, cross-sectional observational studies, and excludes reviews of mixed methods studies. There is no time exclusion on publication dates.

The final search results were exported into Endnote® reference management software, and duplicates were removed. The process was repeated for subsequent databases and sources, with articles sorted into folders and details captured as per the PRISMA flow chart. These results were supplemented by articles found using methods such as citation searching of relevant articles, snowballing and reference list searching.

The titles and abstracts of all papers were screened and performed article selection according to the inclusion and exclusion criteria. Seven papers were identified from the literature search. They are listed below:

| | | | |
|---|---|---|---|
| Akkerman, R.; Logtenberg, M. J... | 2021 | Chicory inulin enhances fermentation of 2'-fucosyllactose by infant fecal microbiota and differentially influ... | Food Funct |
| Goehring, K. C.; Marriage, B. J.; ... | 2016 | Similar to Those Who Are Breastfed, Infants Fed a Formula Containing 2'-Fucosyllactose Have Lower Inflam... | J Nutr |
| Janas, B; Wernimont, S; Gosoni... | 2015 | Clinical safety of a new starter infant formula containing 2 human milk oligosaccharides (HMOs), complete r... | Nestlé Nutrition R&D Clinic... |
| Kajzer, Janice; Oliver, Jeffery; ... | 2016 | Gastrointestinal tolerance of formula supplemented with oligosaccharides | The FASEB Journal |
| Kong, C.; Faas, M. M.; de Vos, P.; ... | 2020 | Impact of dietary fibers in infant formulas on gut microbiota and the intestinal immune barrier | Food Funct |
| Marriage, B. J.; Buck, R. H.; Goe... | 2015 | Infants Fed a Lower Calorie Formula With 2'FL Show Growth and 2'FL Uptake Like Breast-Fed Infants | J Pediatr Gastroenterol Nutr |
| Vandenplas, Y.; de Halleux, V.; A... | 2020 | A Partly Fermented Infant Formula with Postbiotics Including 3'-GL, Specific Oligosaccharides, 2'-FL, and Mi... | Nutrients |

In infants aged less than 8 days of life at inclusion, Kajzer et al. (2016) showed excellent tolerance as evidenced through more consistent softer stools, less reflux and formula intake when fed of a formula supplemented with 2′-FL and FOS for 1 month. This article was an abstract from the Experimental Biology 2016 Meeting. There is no full text article associated with this abstract published in The FASEB Journal.

Kong et al. (2020) produced a table summarising clinical studies undertaken into the effects of infant formula derived dietary fibres on infant health and gut microbiome. The table is partially reproduced below as Table 2. The main findings from studies showed that supplementation of infant formula with 2'-FL and/or GOS/ITF was safe, well tolerated and supported normal growth. It significantly increased *Bifidobacterium* and *Lactobacillus* counts and lowered some inflammatory markers.

Akkerman et al. (2021) concluded from their study that immune compromised infants may benefit from added chicory inulin and 2'-FL in infant formula through immune system modulation effects from improved gut microbiota.

In a randomised, double-blinded, controlled growth and tolerance study on infants fed 2'-FL and GOS, Goehring et al. (2016) concluded that infants fed formula with supplemented 2'-FL exhibit lower plasma and ex vivo inflammatory cytokine profiles, similar to those of a breastfed reference group.

The studies of Marriage et al. (2015) and Vandenplas et al. (2020) are described in section C.3.

The title of Janas et al. (2015) was not able to be accessed for review and was therefore not included.

Table 2: Effects of infant formula derived dietary fibres on the infant health and gut microbiota in clinical trials. Reproduced in part from Kong et al. (2020)

Table 1. Effects of infant formula derived dietary fibers on the infant health and gut microbiota in clinical trials.

| Dietary fibers | Dosing and experiment design | Main findings | Adverse effects | Reference |
|---|---|---|---|---|
| 2′-FL | Infant formula with only galacto-oligosaccharides (GOS) or GOS with 2′-fucosyllactose (2'-FL) of 0.2 g/L or 1 g/L, human milk served as a reference | All formula groups showed similar growth of the infants, and 2'-FL absorption was similar to human milk group. | Not observed | 110 |
| 2′-FL | Infant formula with only GOS or GOS with 2'-FL of 0.2 g/L or 1.0 g/L, breastfeeding as a reference | 2'-FL supplement showed lower inflammatory cytokines, which is similar to breastfeeding group. | Not observed | 111 |
| 2′-FL | Cow's milk based infant formula with or without 2'-FL of 1.0 g/L | 2'-FL in infant formula is safe, well-tolerated, supports growth, and is associated with lower parent-reported morbidity, bronchitis in particular. | Not observed | 112 |
| GOS | Infant formula supplemented with GOS of 4.4 g/L, formula without GOS served as control | Formula supplemented with GOS significantly increased *Bifidobacterium* counts, lowered stool pH, lowered butyric acid concentration. | Not observed | 32 |
| GOS | Infant formula supplemented with GOS (OM55N) of 3 g/L, formula without GOS served as control | Formula supplemented with GOS significantly increased the abundance of *Bifidobacteria* | Not observed | 123 |
| GOS | Iron-containing micronutrient powders with 5 mg iron or 5 mg iron with 7.5 g GOS daily, powder without iron or GOS served as control | Formula supplemented with iron or iron with GOS significantly decreased anaemia of infants. The addition of GOS relieved the adverse effects of iron, i.e. lower abundance of *Bifidobacterium* and *Lactobacillus*. | Not observed | 124 |
| FOS | Infant formula supplemented with or without 1.5 g/L or 3.0 g/L fructo-oligosaccharides (FOS), breastfeeding served as a reference | Formula supplemented with FOS of 1.5g/L resulted in more *Bifidobacteria* counts after 7 days compared to breastfeeding and 3.0 g/L FOS group. | Formula supplemented with FOS of 1.5g/L increased *Clostridium* counts after 7 days. | 136 |
| FOS | Infant formula supplemented with or without 2.0 g/L or 3.0 g/L FOS, breastfeeding served as a reference. | Formula feeding group resulted in a similar abundance of *Bacteroides*, *Bifidobacterium*, and *Lactobacillus* as breastfeeding. | Formula resulted in a higher abundance of *C. difficile* and *E. coli.* | 137 |
| FOS | Infant formula supplemented with 4.0 g/L FOS, 4.0 g/L maltodextrins served as control. | Formula supplemented with FOS of 4 g/L resulted in higher *Bifidobacteria* counts after 2 months of age and higher IgA at 4 months of age. | Not observed | 141 |
| Inulin | Infant formula supplemented with or without 2.0 g/L or 4.0 g/L inulin. | Formula with Inulin of 4 g/L resulted in more *Bifidobacteria* and *Lactobacilli* and lower pH. | Not observed | 135 |
| scGOS/lcFOS/ pectin | Infant formula with only 2 g/L acidic oligosaccharides pectin or a combination of 6 g/L of neutral scGOS/lcFOS mixture and 2 g/L pectin, maltodextrin served as control | The combination of scGOS/lcFOS/pectin resulted in higher abundance of *Bifidobacteria* and *Lactobacilli*, than only pectin and control. | Not observed | 160 |

Two additional papers were identified. Wang et al. (2021) which reported a partially hydrolysed formula (pHF) with GOS/ITF and probiotic (a synbiotic mixture of scGOS/lcFOS (9:1) and *Bifidobacterium breve* M-16V) supported adequate growth and was well tolerated in healthy, term-born Chinese infants. Additionally, infant growth and gastrointestinal tolerance measures of both IF groups were comparable to the breastfed group and was considered suitable and well tolerated for use. Parschat et al. (2021) reported the first multicentre, randomised, controlled, parallel-group clinical study using a cocktail mixture of 5HMOs (5.75 g/L total, comprising 52% 20 -fucosyllactose, 13% 3- fucosyllactose, 26% lacto-N-tetraose, 4% 30 -sialyllactose, and 5% 60 -sialyllactose) added to infant formula. The 5HMO mixture consisted of the five most abundant HMOs at concentrations and ratios resembling those found in breastmilk. The authors concluded that the 5HMO mix at a level of 5.75 g/L total was safe and well tolerated in healthy term infants, with those fed the 5HMOs and the breastfed group producing softer stools at a higher frequency than of the control fed group.

C.5.2 GOS/ITF

A further literature search was conducted to update information available to P306 in 2008 on the safety of GOS/ITF in infant formula products and any studies with GOS/ITF to infant formula products.

This scoping review was planned and conducted using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines extension for scoping reviews (PRIMSA-ScR) (Moher et al. 2015). The aim was to address the following question: “What is known about the safety and health outcomes of adding galactooligosaccharides (GOS) and fructooligosaccharides (FOS) or inulin-type fructans (ITF) to infant formula preparations?”

A series of comprehensive systematic electronic literature searches was performed to find relevant studies reporting on the safety of adding GOS and FOS or ITF to infant formula products.

Recommended health and nutrition electronic data-bases were searched on 30 November 2021 at 14:00 AEST. These databases included: Scopus, Web of Science, Global Health (CABI) and Medline (incl. PubMed). The following search strategy was applied to terms listed within the titles, abstracts and keywords of articles: “(infant formula) AND ((galactooligosaccharides AND fructooligosaccharides OR inulin-type fructans))”.

The review included original research papers that reported on investigations done on human subjects, published as full-length articles in English. It excluded conference abstracts, editorials, letters to the editor and case reports. It included randomised controlled trials, observational cohort studies, cross-sectional observational studies, and excluded reviews of mixed methods studies. There was a time exclusion placed on publication dates, namely: 2008 to 2021.
The final search results were exported into Endnote® reference management software, and duplicates were removed. The process was repeated for subsequent databases and sources, with articles sorted into folders and details captured as per the PRISMA flow chart. These results were supplemented by articles found using methods such as citation searching of relevant articles, snowballing and reference list searching.

The titles and abstracts of all papers were screened, and article selection performed according to the inclusion and exclusion criteria. 26 articles were identified from the literature search. They are listed below:

| Author | Year | Title | Journal |
|---|---|---|---|
| Abrahamse-Berkeveld, M.; Alle... | 2016 | Infant formula containing galacto-and fructo-oligosaccharides and Bifidobacterium breve M-16V supports ... | J Nutr Sci |
| Arslanoglu, S.; Moro, G. E.; Sch... | 2008 | Early dietary intervention with a mixture of prebiotic oligosaccharides reduces the incidence of allergic ma... | J Nutr |
| Béghin, L.; Tims, S.; Roelofs, M.;... | 2021 | Fermented infant formula (with Bifidobacterium breve C50 and Streptococcus thermophilus O65) with pre... | Clin Nutr |
| Bellaiche, M.; Ludwig, T.; Arcisz... | 2021 | Safety and Tolerance of a Novel Anti-Regurgitation Formula: A Double-Blind, Randomized, Controlled Trial | J Pediatr Gastroenterol Nutr |
| Bocquet, A.; Lachambre, E.; Ke... | 2013 | Effect of infant and follow-on formulas containing B lactis and galacto- and fructo-oligosaccharides on infe... | J Pediatr Gastroenterol Nutr |
| Chouraqui, J. P.; Grathwohl, D.; ... | 2008 | Assessment of the safety, tolerance, and protective effect against diarrhea of infant formulas containing m... | Am J Clin Nutr |
| Christides, T.; Ganis, J. C.; Sharp,... | 2018 | In vitro assessment of iron availability from commercial Young Child Formulae supplemented with prebiotics | Eur J Nutr |
| Chua, M. C.; Ben-Amor, K.; Lay, ... | 2017 | Effect of Synbiotic on the Gut Microbiota of Cesarean Delivered Infants: A Randomized, Double-blind, Mult... | J Pediatr Gastroenterol Nutr |
| Closa-Monasterolo, R.; Gispert-... | 2013 | Safety and efficacy of inulin and oligofructose supplementation in infant formula: results from a randomize... | Clin Nutr |
| Comstock, S. S.; Li, M.; Wang, M... | 2017 | Dietary Human Milk Oligosaccharides but Not Prebiotic Oligosaccharides Increase Circulating Natural Killer ... | J Nutr |
| da Costa Ribeiro, H. Júnior; Rib... | 2015 | Normal Growth of Healthy Infants Born from HIV+ Mothers Fed a Reduced Protein Infant Formula Containi... | Clin Med Insights Pediatr |
| González, R.; Klaassens, E. S.; M... | 2008 | Differential transcriptional response of Bifidobacterium longum to human milk, formula milk, and galactool... | Appl Environ Microbiol |
| Holscher, H. D.; Faust, K. L.; Cze... | 2012 | Effects of prebiotic-containing infant formula on gastrointestinal tolerance and fecal microbiota in a rando... | JPEN J Parenter Enteral Nutr |
| Huet, F.; Abrahamse-Berkeveld... | 2016 | Partly Fermented Infant Formulae With Specific Oligosaccharides Support Adequate Infant Growth and Ar... | J Pediatr Gastroenterol Nutr |
| Kongnum, K.; Taweerodjanakar... | 2020 | Impacts of Prebiotic-Supplemented Diets and Breastmilk on Population and Diversity of Lactobacilli Establis... | Curr Microbiol |
| Kosuwon, P.; Lao-Araya, M.; Uth... | 2018 | A synbiotic mixture of scGOS/lcFOS and Bifidobacterium breve M-16V increases faecal Bifidobacterium in ... | Benef Microbes |
| Lee Ie, Y.; Bharani, R.; Biswas, A.... | 2015 | Normal growth of infants receiving an infant formula containing Lactobacillus reuteri, galacto-oligosacchari... | Matern Health Neonatol Pe... |
| Neumer, F.; Urraca, O.; Alonso, J... | 2021 | Long-Term Safety and Efficacy of Prebiotic Enriched Infant Formula-A Randomized Controlled Trial | Nutrients |
| Rodriguez-Herrera, A.; Mulder, ... | 2019 | Gastrointestinal Tolerance, Growth and Safety of a Partly Fermented Formula with Specific Prebiotics in He... | Nutrients |
| Salvini, F.; Riva, E.; Salvatici, E.; ... | 2011 | A specific prebiotic mixture added to starting infant formula has long-lasting bifidogenic effects | J Nutr |
| Scholtens, P. A.; Alliet, P.; Raes, ... | 2008 | Fecal secretory immunoglobulin A is increased in healthy infants who receive a formula with short-chain gal... | J Nutr |
| Schouten, B.; Van Esch, B. C.; Ko... | 2011 | Non-digestible oligosaccharides reduce immunoglobulin free light-chain concentrations in infants at risk fo... | Pediatr Allergy Immunol |
| Stam, J.; van Stuijvenberg, M.; Ga... | 2011 | A mixture of three prebiotics does not affect vaccine specific antibody responses in healthy term infants in the fir... | Vaccine |
| Szajewska, H.; Ruszczyński, M.; ... | 2017 | Effects of infant formula supplemented with prebiotics compared with synbiotics on growth up to the age ... | Pediatr Res |
| van Hoffen, E.; Ruiter, B.; Faber,... | 2009 | A specific mixture of short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides induces a ... | Allergy |
| Vandenplas, Y.; de Halleux, V.; A... | 2020 | A Partly Fermented Infant Formula with Postbiotics Including 3'-GL, Specific Oligosaccharides, 2'-FL, and Mi... | Nutrients |

In 25 of the 26 papers identified, all infant formulas tested in the studies reported were associated with normal growth for infants and were well tolerated and are listed in References. Béghin et al. (2021) reported additionally that formula fed to 4-month-old infants showed they had microbiome composition and metabolic activity closer to that of breastfed infants.

Bocquet et al. (2013), whilst reporting normal growth for infants fed GOS/FOS in their study in comparison with WHO growth standards, also reported that formulas containing *B. lactis* and GOS/FOS did not reduce infection rates beyond those that contained *B. lactis* only.

10 of the 26 papers identified reported on the efficacy, safety and tolerance of a specific mixture of GOS/ITF added to infant formula (Abrahamse-Berkeveld et al. 2013, Chua et al. 2017, Huet et al. 2016, Neumer et al. 2021, Rodriguez-Herrera et al. 2019, Salvini et al. 2011, Szajewska et al. 2017, van Hoffen et al. 2009 & Vandenplas et al. 2020). This mixture was the same mixture as used in Nutricia products, permitted in Standard 2.9.1. Of the 10 papers studying the specific GOS/ITF mixture, 3 included the addition of a specific probiotic (Abrahamse-Berkeveld et al. 2013, Chua et al. 2017 & Szajewska et al. 2017). All 10 studies, conducted as double-blind, controlled clinical trials reported the study formula to be safe, well-tolerated and providing satisfactory/similar infant growth, compared to a control infant formula.

Huet et al. (2016) and Vandenplas et al. (2020) reported clinical evidence to support the safety, adequate growth and tolerance of GOS/ITF in healthy infants fed a partially fermented formula. Huet et al. (2016) further confirmed a beneficial effect of scGOS/lcFOS (9:1 ratio) on gut microbiota and stool characteristics in infant formula. Rodriguez-Herrera et al. (2019) reported partly fermented formula with prebiotics (scGOS/lcFOS 9:1 ratio) induced stool consistency closer to breastfed infants. Neumer et al. (2021) also reported softer stools for infants fed with prebiotic formula and higher counts of Bifidobacterium levels in the first six months of life, with associated reduced duration of infections.

Comstock et al. (2017), as the 26$^{th}$ paper identified, was a paper that investigated the effectiveness of dietary HMOs in an animal study. This is included in Section C.3 of this application.

An additional paper identified, Vandenplas et al. (2015), reported the addition of GOS/ITF to be very safe in infant formula and to have a bifidogenic effect, bringing stool consistency and frequency closer to those of breastfed infants.

### C.5.3 Conclusion

The studies identified in the literature searches discussed in sections C.5.1 and C.5.2 confirm FSANZ's earlier assessment outcomes that 2'-FL and GOS/ITF are ingredients that are safe for addition to infant formula products.

## D Information on the dietary intake of the nutritive substance

*(addressing section 3.5.2.D of the FSANZ Application Handbook)*

Information in this section includes proposed uses and anticipated levels of exposure in the relevant population groups. This section addresses the information requirements in the FSANZ Application Handbook for Guidelines 3.3.3 (Nutritive Substances) and 3.6.2 (Special Purpose Foods – Infant Formula Products). The following table sets out which parts of this section address each of the Guideline requirements.

| Handbook guideline | Handbook requirement | Application section |
|---|---|---|
| 3.3.3 – Nutritive substances | D.1 A detailed list of the food groups or foods in which the use of a nutritive substance is proposed | D.1 |
| | D.2 The maximum proposed level of the use of the nutritive substance for each food group or food | D.1 |
| | D.3 For foods or food groups not currently listed in the most recent Australian or New Zealand National Nutrition Surveys (NNSs), information on the likely level of consumption | D.3 |
| | D.4 The percentage of the food group to which the use of the nutritive substance is proposed or the percentage of the market likely to use the nutritive substance | D.4 |
| | D.5 Information relating to the use of the nutritive substance in other countries | D.5 |
| | D.6 For foods where consumption has changed in recent years, information on likely current food consumption | D.6 |
| 3.6.2 – Special purpose foods – Infant formula products | B.1 Data to enable the dietary intake or exposure of the target population to be estimated | D.1, D.3 |
| | B.2 Data on the recommended level of formula consumption for the target population | D.3 |
| | B.3 Information relating to the substance (naturally occurring or added to other foods that infants are likely to consume) | D.2 |

### D.1 List of proposed food groups and maximum levels of use

The combination of GOS/ITF and 2'-FL is to be added to infant formula and follow-on formula, including special dietary use, in order to provide immune benefits that may otherwise be achieved through the consumption of human milk.

Table 3: Proposed uses of GOS/ITF and 2'-FL for infant formula, follow-on formula

| **Specified Food Category** | Maximum Level* | |
|---|---|---|
| | **GOS/ITF** | **2'-FL** |
| Infant formula | 8 g/L | 2.4 g/L |
| Follow-on formula | 8 g/L | 2.4 g/L |

## D.2 Oligosaccharide content of human milk and infant formula products

Pre-application discussions with FSANZ identified that the oligosaccharide load of infant formula containing 2'-FL at up to 2.4 g/L and GOS/ITF at up to 8 g/L should be considered in the context of the oligosaccharide load of human milk. The intended use of 2'-FL and GOS/ITF in infant formula and follow-on formula will therefore result in a total 2'-FL and GOS/ITF content of 10.4 g/L of these oligosaccharides. This is less than the reported average oligosaccharide load of human milk.

The oligosaccharide content of human milk was investigated in a systematic review by Thurl et al. (2017). These authors noted that HMO content can vary across individuals, by gestational age and by the lactation period. However, they identified twenty-one studies that reported on HMO content and determined average quantities of HMO reported in studies of term and pre-term milks. The average quantity of neutral HMOs present in term milk of secretor mothers was 14.78 g/L with a total concentration of all HMOs at approximately 17 g/L. These levels were higher than pre-term milk (average = 11.57 g/L). This compares favourably to the levels of HMO in mature human milk of 10-15 g/L reported by Jantscher-Krenn et al. (2012). The 10-15 g/L total HMO range is also consistent in an earlier publication of Kunz and Rudloff (2006).

Health Canada (2020) in its acceptance of addition of the Chr. Hansen (formerly Jennewein) 2'-FL concluded that "as bovine milk contains less oligosaccharides than human milk, and specifically less 2'-FL, infants on un-supplemented bovine milk formulas are currently exposed to less oligosaccharides and 2'-FL than human milk fed infants. The dietary exposure for infants consuming 2'-FL in infant formula would be lower or consistent with current exposure for breastfed infants, as the proposed rate of addition of 2'-FL in formulas is lower than that in breast milk. Breast milk contains on average 2.38 g 2'-FL /L (Erney et al. 2000), which is about two-fold higher than the petitioner's proposed maximum specification of 1.2 g 2'-FL /L (includes overage)".

## D.3 Information on likely level of consumption (foods not in ANZ NNSs)

Food consumption data is not available for children under two years of age in Australian NNSs or for children under five years of age in New Zealand NNSs. As part of P306 (FOS & GOS Final), FSANZ constructed model diets for infants aged three, nine and twelve months. In New Zealand, a model diet was constructed for toddlers aged one to three years, however this is not relevant to the intended use of GOS/ITF and 2'-FL in this application. These infant diet models included an estimated intake of 800 mL/day of infant formula for three-month-olds, 545 mL/day of follow-on formula for nine-month-old infants and 425 mL/day of follow-on formula for twelve-month-old infants.

Based on these model diets and intended use levels of GOS/ITF and 2'-FL in this application, estimated dietary intakes have been calculated and presented in Table 4. The table includes the estimated intakes of 2'-FL and GOS/ITF for 12 month old infants. This is considered relevant for inclusion as a 12 month old infant is the upper end of the age range for recommended consumption for infant formula products. Please note that toddler milks are not included as a proposed food in this application.

Table 4: Estimated intake of 2'-FL and GOS/ITF based on FSANZ model infant diets

| Age | Infant formula intake | Projected intake | Increase* |
|---|---|---|---|
| 3 months | 800 mL | 6.4 g/day GOS/ITF<br>0.8 – 1.92 g/day 2'-FL # | No change<br>0.8 – 1.92 g/day 2'-FL |
| 6 months | 800 mL | 6.4 g/day GOS/ITF<br>0.8 g/day – 1.92 g/day 2'-FL # | No change<br>0.8 – 1.92 g/day 2'-FL |
| 9 months | 545 mL | 4.36 g/day GOS/ITF<br>0.55 g/day – 1.31 g/day 2'-FL # | No change<br>0.55 g/day – 1.31 g/day 2'-FL |
| 12 months | 425 mL | 3.4 g/day GOS/ITF<br>0.43 g/day – 1.02 g/day 2'-FL | No change<br>0.43 g/day – 1.02 g/day 2'-FL |

* Already have GOS/ITF in market

# Assuming range of 2'-FL goes to 2.4 g/L (as per A1190 approval). Range 1.0 – 2.4 g/L 2'-FL.

Source: FSANZ estimates based on model diets for each age group developed under Proposal P306.

Guideline 3.6.2.B.2 of the Application Handbook requires information to be provided on the recommended level of infant formula consumption. The following data relates to Nutricia's Aptamil brand of infant formula:

*Stage 1 (0-6months)*

| | |
|---|---|
| The capacity of the product | 900 g |
| The number of scoops required per feed<br><br>50ml cooled boiled water per scoop of product<br>Scoop weight 7.3g | Stage 1 (0-6 Months) |

| Age | Cooled boiled water | Scoops | Feeds per day |
|---|---|---|---|
| Birth to 2 weeks | 50 mL | 1 | up to 10 |
| 2 - 4 weeks | 100 mL | 2 | 6-7 |
| 1-2 months | 150 mL | 3 | 5-6 |
| 3-4 months | 150 mL | 3 | 5-6 |
| 5-6 months | 200 mL | 4 | 4-5 |

| | |
|---|---|
| The volume of water required per feed | As per above calculation |
| Total volume of the made-up feed | As per above calculation |
| Recommended number of feeds per day relevant to each age group in the relevant target population | As per above calculation |

*Stage 2 (6-12months)*

| | |
|---|---|
| The capacity of the product | 900 g |
| The number of scoops required per feed<br><br>50ml cooled boiled water per scoop of product<br>Scoop weight 7.5g | Stage 2 (6-12 Months) |

| Age | Cooled boiled water | Scoops | Feeds per day |
|---|---|---|---|
| 6-8 months | 200 mL | 4 | 3-5 |
| 9-12 months | 200 mL | 4 | 3-4 |

| | |
|---|---|
| The volume of water required per feed | As per above calculation |
| Total volume of the made-up feed | As per above calculation |
| Recommended number of feeds per day relevant to each age group in the relevant target population | As per above calculation |

## D.4 Percentage of food group to which the nutritive substance is proposed to be added

Based on current estimated market share assumptions in Australia as at 30 November 2021 it would be estimated that around 25% (Nutricia Aptamil Brand) of the total baby formula market would contain the nutritive substances (2'-FL + GOS/ITF) if permitted to be added. Other brand IFP have only ITF added (this accounts for a low percentage of market share) and the approved 2'-FL from A1155 is added to IFP now available in Australia as at 30 November 2021. A current application (A1233, December 2021) for approval of Friesland-Campina 2'-FL may also increase the volume of products available on the ANZ market containing 2'-FL in the future.

## D.5 Information relating to the use of the nutritive substance in other countries

Nutricia have launched infant formula products that contains 2'-FL and scGOS/lcFOS (9:1 ratio) combination in the following countries worldwide:

Table 5: Countries where Nutricia has launched 2'-FL and scGOS/lcFOS combination infant formula products

| Country | Launch Date | Age Range | 2'-FL max use level | scGOS/lcFOS max use level |
|---|---|---|---|---|
| Portugal | May 2021 | 0-6 months<br>6-12 months | 1.2 g/L | 8 g/L |
| Germany | April 2021 | 0-6 months<br>6-12 months | 1.2 g/L | 8 g/L |
| Romania | Jan 2021 | 0-6 months<br>6-12 months | 1.2 g/L | 8 g/L |
| Belgium | Dec 2020 | 0-6 months<br>6-12 months<br>1 years+ | 1.2 g/L | 8 g/L |
| United Kingdom | Dec 2020 | 0-6 months<br>6-12 months<br>1 years+<br>2 years+ | 1.2 g/L | 8 g/L |
| Austria | Nov 2020 | 0-6 months<br>6-12 months<br>1 years+<br>2 years+ | 1.2 g/L | 8 g/L |
| Thailand | Nov 2020 | 0-3 years<br>> 3 years | 1.0 g/L<br>1.2 g/L | 8 g/L<br>8 g/L |
| Greece | Oct 2020 | 0-6 months<br>6-12 months | 1.2 g/L | 8 g/L |
| Netherlands | Sept 2020 | 0-6 months<br>6–12 months | 1.2 g/L | 8 g/L |
| Italy | Aug 2020 | 0-6 months<br>6-12 months<br>1 year + | 1.2 g/L | 8 g/L |
| Ireland | July 2020 | 0-6 months<br>6-12 months<br>1 years+ | 1.2 g/L | 8 g/L |
| Hong Kong | May 2020 | 0-6 months<br>6-12 months<br>1-3 years<br>3 years+ | No max level in Regulation (regarded as safe) | No max level in Regulation (regarded as safe) |
| Poland | Feb 2020 | 0-6 months | 1.2 g/L | 8 g/L |

| Country | Launch Date | Age Range | 2'-FL max use level | scGOS/lcFOS max use level |
|---|---|---|---|---|
| | | 6-12 months<br>1-2 years<br>2 years+ | | |
| Spain | Feb 2020 | 0-6 months<br>6-12 months | 1.2 g/L | 8 g/L |
| Slovakia | Dec 2019 | 0-6 months<br>6-12 months<br>1-2 years<br>2 years+ | 1.2 g/L | 8 g/L |
| Czech Republic | Dec 2019 | 0-6 months<br>6-12months<br>1-2 years<br>2 years+ | 1.2 g/L | 8 g/L |
| Switzerland | Oct 2019 | 0-6 months<br>6-12 months | 1.2 g/L | 8 g/L |

Additionally, 2'-FL, GOS, ITF are used individually or in some combination in the EU, USA, South America and Asian countries in infant formula products manufactured by other companies.

In A1190 (Approval Report, p10), Chr.Hansen confirmed that product containing its 2'-FL had been launched in almost 30 countries at a range of 1.0 -2.0 g/L.

### D.6 Foods where consumption has changed – information on likely consumption

Nutricia and Chr. Hansen are not aware of any data to suggest that the consumption of infant formula products has changed or will change significantly in Australia and New Zealand in recent and coming years.

### D.7 Dietary Exposure Conclusions

Human milk oligosaccharides (HMOs) are a pool of complex carbohydrates. Currently, approximately 160 oligosaccharide structures in human milk have been elucidated, and many more are present, at least in small quantities. There are many different functions attributed to HMOs, which may explain the great variety of their structures. Assuming a maximum human milk intake of 0.8 L/day and an oligosaccharide content of 5-15 g/L, a breastfed infant would consume between 4 - 12 g per day of non-digestible oligosaccharides during the first half year of life (EFSA 2014). Moreover, a recent meta-analysis of HMOs' concentration has revealed a total of approximately 17 g/L of HMOs in secretor milks (Thurl et al. 2017), whereas in colostrum, the concentrations of oligosaccharides can even go up to 20 g/L or more (Gabrielli et al. 2011).

Nutricia manufactures infant milk products that contain a unique blend of oligosaccharides consisting of short-chain galacto-oligosaccharides (scGOS) and long-chain fructo-oligosaccharides (lcFOS) in a ratio of 9:1, which is similar to the ratio of HMOs in human milk, and is added in an amount of 8 g/L. This level reflects the quantity of HMOs most closely to the ones in breast milk. The prebiotic mixture scGOS/lcFOS contains around 100 different oligosaccharides structures and mimics in this way the diversity of the pool of HMOs. This prebiotic mixture of scGOS/lcFOS not only mimics the diversity of HMOs structures and the short- and long-chain ratio of HMOs, but also resembles the prebiotic effects and the functional benefits of HMOs. The beneficial effects of the prebiotic mixture scGOS/lcFOS are supported by

a strong body of evidence with more than 40 clinical studies in over 90 publications (Salminen et al. 2020). Salminen et al. (2020), in a review of clinical research on scGOS/lcFOS (9:1) showed benefits linked to modulation of the gut microbiota and the immune system, reduced incidence of infections, and stool softening. This prebiotic mixture has been recognised as a prebiotic by The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics (Gibson et al. 2017).

2'-FL is the most abundant oligosaccharide in human milk, serving as a substrate for commensal gut bacteria (Yu et al. 2012). While the levels of 2'-FL varies throughout the lactation period and per individual, the overall range found in human milk from secretors is 0.6 – 7.8 g/L. Estimates of dietary intakes of 2'-FL based on a concentration of 2.4 g/L for infants up to 12 months ranged between 0.1 – 0.33 g/kg bw/day at the mean and 0.2 – 0.66 g/kg bw/day at the 90$^{th}$ percentile, and for children 2-3 years from 0.077 – 0.15 g/kg bw/day at the mean and 0.15 – 0.31 at the 90$^{th}$ percentile. These ranges reflect typical intakes of infants and young children who receive human milk from mothers who are secretors.

FSANZ completed a dietary intake assessment of 2'-FL for A1155 (Approval Report, p40) and concluded that an additional dietary intake assessment was not required for A1190. The estimated dietary intake of 2′-FL based on 2.4 g/L in infant formula and follow-on formula, was similar to 2'-FL intakes for three and nine month old, breastfed infants, with twelve month old infants similar to or less than those for younger formula-fed and breastfed infants (< 12 months).

In a double-blind, randomised, controlled, parallel group, multi-country study trial (Vandenplas et al. 2020), healthy fully infant formula (IF)-fed infants were randomised ≤14 days of age to one of two formulas until 17 weeks of age:

Test: IF with scGOS/lcFOS (9:1; 8 g/L), 2'-FL (1 g/L)
Control: IF with scGOS/lcFOS (9:1; 8 g/L)

In this study equivalence in weight gain (primary outcome) between the test and control groups was confirmed, as well as equivalence in length and head circumference gain. No statistically significant differences were observed in adverse events or gastrointestinal tolerance between randomised infant formula groups. It was concluded that this infant formula product with a total 9 g/L of oligosaccharide, (scGOS/lcFOS (9:1) and 2'-FL), supported adequate infant growth and was safe and well-tolerated in healthy term infants.

# E INFORMATION ON THE NUTRITIONAL IMPACT OF THE NUTRITIVE SUBSTANCE

This section addresses Application Handbook requirements relating to the nutritional and health impact of the combination of GOS/ITF and 2'-FL in infant formula products. These requirements span the more general requirements for nutritive substances and the specific requirements for special purpose foods. The relevant handbook requirements and how these have been addressed in this section of the application are highlighted in the table below.

| Handbook Guideline | Requirement | Application section |
|---|---|---|
| 3.3.3 – Nutritive substances | F.1 Information related to the nutritional purpose of each substance in food | Section E.1 |
| 3.6.2 – Special purpose foods – Infant formula products | A.1 Purpose of the compositional change | Section E.1 |
| | A.2 General data requirements for supporting evidence | |
| | A.3 Specific information requirements of the nutritional safety, tolerance and efficacy of the proposed compositional change: | Section C |
| | Characterisation of proposed substance or the comparable substances in breast milk | Section B, C |
| | Nutritional safety and tolerance of the proposed compositional change | Section C |
| | Efficacy of the proposed compositional change | Section E.1 |

## E.1 Purpose of the compositional change

The addition of both 2'-FL and GOS/ITF to infant formula and follow-on formula at the intended levels (up to 2.4 g/L and 8 g/L respectively) is consistent with average levels of HMO present in human milk and is intended to provide formula-fed infants with comparable oligosaccharide intake levels to those of breastfed infants. The oligosaccharide load of Nutricia's infant formula products is therefore also likely to be less than the average oligosaccharide content of human milk and is therefore not expected to cause a nutritional imbalance in the diet of infants. The safety of addition of a combination of 2'-FL and GOS/ITF is described in section C.

Clinical studies have shown that supplementation of infant formula with a specific prebiotic mixture (such as short-chain galacto-oligosaccharides (scGOS) and long-chain fructo-oligosaccharides (lcFOS) (9:1)), a ratio mimicking the diversity of oligosaccharides present in human milk, leads to a more favourable gut microbiota composition and activity, closer to that observed in breastfed infants (Vandenplas et al. 2020). GOS and ITF in infant formula has been associated with a lower number of infections, fever episodes, and antibiotic prescriptions (Salminen et al. 2020). The addition of HMOs such as 2'-FL to infant formula has been suggested to result in lower inflammatory markers and fewer parental-reported bronchitis and respiratory tract infections.

Prebiotic mixtures of GOS/ITF mimic the high diversity of HMOs structures by providing more than 100 different structures. 2'-FL is the most abundant HMO present in human milk and the 2'-FL structure of permitted forms in the Code is identical to the structure found in human milk. The concept of the combination with GOS/ITF and 2'-FL therefore resembles the complex HMOs composition in human milk more closely than GOS/ITF or 2'-FL individually.

The concept with GOS/ITF and 2'-FL also mimics the prebiotic effects and functional benefits of human milk more closely. The proven clinical studies beneficial effects of GOS/ITF and 2'-FL demonstrated over the past several decades are maintained in combination, including modulating the infant gut microbiota through stimulation of higher proportions of *Bifidobacterium* in relation to total gut bacteria, production of softer stool consistency, decreasing rates and duration of infant infections and reduction in febrile episodes, overall moderating certain functions of the immune system (Rodriguez-Herrera et al. 2019, Vandenplas et al. 2020, Neumer et al. 2021)

Data from first *in vivo* pre-clinical investigations showed positive effects of the combination of scGOS/lcFOS and 2'-FL. In a rotavirus (RV) model, anti-diarrhea effects of scGOS/lcFOS and 2'-FL was studied. scGOS/lcFOS, 2'-FL and scGOS/lcFOS/2'-FL combined all reduced RV-induced diarrhea. However, the mechanisms involved differed. scGOS/lcFOS had the highest RV-blocking effect *in vitro*, whereas 2'-FL had the strongest effect on pathogen recognition receptors (Azagra-Boronat et al. 2018).

In addition, the combination of scGOS/lcFOS and 2'-FL in an *in vivo* vaccination model enhanced the influenza vaccine responses which are associated with mucosal immune regulation. The observed immunological changes were correlated with microbial community structure and metabolites (Van den Elsen et al. 2019; Xiao et al. 2019).

Furthermore, Overbeek et al. (2019) showed that 2'-FL in the presence of scGOS/lcFOS was more effective in modulating dendritic cell maturation *in vitro*, compared to 2'-FL alone.

In another *in vitro* model in which a simulator of the human intestinal microbial ecosystem (SHIME®) was used, the impact of the specific combination of scGOS/lcFOS and 2'-FL on the eco-physiology of the gut in early life was investigated. In this experiment it was shown that 2'-FL was only fermented in the presence of scGOS/lcFOS resulting in a bacterial composition suggested to confer health benefits. These results suggest that infants with a specific gut microbiome composition might not be able to benefit solely from 2'-FL, yet in the presence of scGOS/lcFOS it could confer more beneficial effects (Goh et al. 2019).

In summary, combining GOS/ITF and 2'-FL mimics the pool of HMOs more closely with respect to diversity in structures, short and long-chain ratio and it contains one identical structure as found in human milk. The combination mimics HMOs in human milk more closely with respect to prebiotic effects and functional benefits.

### E.2 Information related to the nutritional impact of the use of the substance in each food

The nutritional impact of the individual ingredients added to infant formula has previously been assessed in detail by FSANZ. Chr. Hansen 2'-FL in A1190 (Approval Report) and in the case of GOS/ITF in P306 (FOS & GOS FAR FINAL). These assessments concluded that the individual ingredients did not indicate a nutritional concern at the concentrations used. Since P306 (2008), there has been no reports to indicate any additional nutritional concerns. When 2'-FL and GOS/ITF are combined, there are no additional concerns raised, with no deleterious effect on growth profiles of infants.

Further investigation on prebiotics in the food supply has continued since P306 was published in 2008. Prebiotics are naturally present in a variety of foods such as fruit, vegetables, wholegrains and pulses, consumption of which is encouraged in national and international dietary guidelines

(Australian Government Department of Health 2017). The prebiotic content of a range of foods, derived from analysis carried out in Australia using consistent methodology, can be seen in Table 6.

Table 6: Prebiotic content of selected foods (adapted from Lockyer & Stanner (2019))

| Food source | GOS* (g/100 g food as eaten) | FOS[ł] (g/100 g food as eaten) | Total fructans[‡] (g/100 g food as eaten) |
|---|---|---|---|
| Rye bread | 0.24 | ND | 1.05 |
| White bread | 0.20 | 0.11 | 0.68 |
| Wholegrain bread | 0.59 | ND | 0.69 |
| Bran-based cereal | 1.32 | 0.66 | 2.35 |
| Cornflakes | | ND | 1.07 |
| Muesli | 0.34 | ND | 1.26 |
| Oats | 0.34 | ND | 0.32 |
| Puffed rice cereal | ND | ND | 1.04 |
| Wheat biscuits cereal | 0.31 | ND | 2.05 |
| Cereal bar (with dried fruit) | ND | ND | 2.53 |
| Crackers | ND | ND | 0.77 |
| Potato crisps | ND | ND | 0.22 |
| Pretzels | ND | 0.48 | 1.40 |
| Rice cakes | TR | ND | 1.35 |
| Rye crispbread | ND | 1.95 | 4.60 |
| Shortbread biscuit | ND | ND | 1.25 |
| Brown Rice | ND | ND | TR |
| Cous cous | ND | ND | 0.73 |
| Gnocchi | ND | ND | 0.60 |
| Pasta | ND | ND | 0.34 |
| White rice | ND | ND | ND |
| Butter beans (canned) | 0.42 | 0.22 | 0.14 |
| Chickpeas (canned) | 0.19 | 0.07 | 0.16 |
| Lentils (canned) | 0.22 | | 0.15 |
| Red kidney beans (boiled) | 1.44 | 0.51 | 0.54 |
| Asparagus | ND | 0.43 | 0.0 |
| Beetroot | 0.14 | 0.33 | 0.4 |
| Broccoli | 0.13 | 0.79 | ND |
| Garlic | ND | 0.92 | 17.4 |
| Onion (white) | 0.19 | 0.39 | 1.8 |
| Blueberries | ND | 0.44 | ND |
| Nectarine | ND | 0.59 | 0.21 |
| Pineapple | ND | 0.10 | TR |
| Raspberries | ND | 0.30 | TR |
| Watermelon | ND | 0.20 | 0.32 |

Source: Data derived from studies carried out in Australia (Muir et al. 2007, 2009; Biesedierski et al. 2011).
GOS, galacto-oligosaccharides: FOS, fructo-oligosaccharides: ND, not detectable; TR, trace amounts detected
N.B. Values for 'total fructans' differ to values for 'FOS' as foods contain fructans of different chain lengths, aside from nystose and kestose.
*Raffinose plus stachyose, measured via high-performance liquid chromatography (HPLC) with an evaporative light scattering detection (ELSD).
[ł]Nystose plus kestose, measured via HPLC with ELSD.
[‡]including all FOS and inulin, measured via enzymic assay.

A review by the UK Scientific Advisory Committee on Nutrition (SACN) in 2015 resulted in a change to UK dietary reference values for dietary fibre. The value increased from ~24 g/ day to 30 g/day for adults (SACN 2015). Lockyer & Stanner (2019) reported that average intakes in the UK were well below this at ~19 g/day for adults. Higher dietary reference values were also set for children (15 g/day for children aged 2–5 years, 20 g/day for children aged 5–11 years, 25 g/day for children aged 11–16 years and 30 g/day for adolescents aged 16–18 years). These new values are also not being met by the majority of children, signalling a need to increase intake in these age groups.

Table 7: Dietary reference values for, and intakes of, dietary fibre in the UK. Adapted from Lockyer & Stanner (2019)

| Age group (years) | Dietary recommendation (g/day) | Mean intake – males (g/day) | Mean intake – females (g/day) |
|---|---|---|---|
| 1.5-3 | 15 | | 10.3* |
| 4-10 | 20 | 14.5 | 13.5 |
| 11-18 | 25 | 16.5 | 14.1 |
| 19-64 | 30** | 20.7 | 17.4 |
| 64+ | 30 | 19.0 | 16.4 |
| 75+ | 30 | 18.3 | 15.1 |

Sources: SACN (2015; Roberts et al. (2018).
*Mean intake for all children.
**Dietary reference value for adolescents aged 16-18 years is 30 g/day.

The intake of specific prebiotics like GOS/ITF via complementary feeding for infants 0-12 months is limited. For infants 0-6 months, feeding infant formula is regarded as a sole source of nutrition up to after 4 months of age, or as complementary to breast milk. Therefore under 4 months of age, the intake from other foods is zero. As an infant starts complementary feeding with other foods from approximately 4 months of age (certainly from 6 months of age), the estimated intake of GOS/ITF is considered to be <2 g per day (based on estimated intake in adults of GOS/ITF at 2-4 g per day). In clinical intervention studies with scGOS/lcFOS the natural dietary intake of 0–12-month infants was not corrected for due to it being so low. In the early study of Scholtens et al (2006), considered in P306, in which GOS/ITF was added to weaning food, the potential intake from GOS/ITF from the rest of the diet was not considered.

SACN (2015) additionally reported strong evidence from observational studies that suggests significant associations between high-fibre diets and reduced risk of chronic diseases (i.e. cardiovascular disease (CVD), type 2 diabetes and colorectal cancer). SACN reported that randomised controlled trials (RCTs) provided some evidence into some mechanisms behind these links, where consuming more dietary fibre decreases intestinal transit times, increases faecal bulk and decreases constipation. This highlighted that particular fibre types were shown to “decrease total cholesterol, low-density lipoprotein (LDL) cholesterol and triacylglycerol concentrations, lower blood pressure, reduce postprandial glycaemia and change faecal bacterial content” (SACN 2015).

ITF and GOS continue to be widely accepted as prebiotics (Gibson et al. 2017), and several other ‘candidate’ prebiotics have been described. Candidate prebiotics are food components

that have demonstrated prebiotic potential in vitro or in animals but for which sufficient data from human studies are lacking (Table 8) (Lockyer & Stanner 2019).

Further evidence since P306 (2008) is available for ITF and GOS demonstrating a role in increasing bifidobacteria in the human gut (SACN 2015; So et al. 2018). This is thought to benefit human health through the displacement of pathogens and modulation of the immune system (Wallace et al. 2011). A recent systematic review and meta-analysis of RCTs by So et al. (2018) confirmed that prebiotics stimulate the growth of Bifidobacterium and Lactobacillus species, whereas general fibre types do not.

Table 8: List of accepted and candidate prebiotics. Adapted from Lockyer & Stanner (2019)

| Accepted prebiotics | Candidate prebiotics* |
|---|---|
| Inulin-type fructans<br>• Inulin [degree of polymerisation 2-60, average 12 (high molecular weight inulin average 25)]<br>• Fructo-oligosaccharides [FOS (fructans of short-chain length, degree of polymerisation 2-8, average 3.6)]<br>• Oligofructose [degree of polymerisation 2-8, average 4]<br>Galactans (degree of polymerisation 2-8)<br>• Galacto-oligosaccharides (GOS)<br>• Trans-galacto-oligosaccharides (TOS) | Polydextrose<br>Soya bean oligosaccharides<br>Lactosucrose<br>Isomalto-oligosaccharides<br>Gluco-oligosaccharides<br>Xylo-oligosaccharides<br>Gentio-oligosaccharides<br>Mannan-oligosaccharides<br>Lactose<br>Hemicellulose<br>Resistant starch<br>Resistant dextrins<br>Oat bran<br>Oligosaccharides from melibiose<br>β-glucans<br>N-acetylchitooligosaccharides<br>Arabinoxylan-oligosaccharides<br>Sugar alcohols (e.g. lactitol, sorbitol and maltitol) |

Sources: Roberfroid et al. (2010); Carlson et al. (2018).
*Described as dietary carbohydrates that show some fermentation selectivity when tested in laboratory systems, but for which evidence from human studies is lacking. List may not be exhaustive; more recently, guar gum, lactulose and resistant maltodextrins have also been suggested as prebiotics (Carlson et al. (2018)).

In a review article by Franco-Robles & López (2015), the authors discussed advances in the understanding of the interactions of fructans with the intestinal immune system to modulate immune responses, stimulate beneficial intestinal bacteria and inhibit the growth and survival of pathogens in the gut.

The addition of both 2'-FL and scGOS/lcFOS to infant formula and follow-on formula at the intended levels (up to 2.4 g/L and 8 g/L respectively) is consistent with average levels of HMO present in human milk and is intended to provide formula-fed infants with comparable oligosaccharide intake levels to those of breastfed infants. The oligosaccharide load of Nutricia's infant formula products is therefore also likely to be less than the average oligosaccharide content of human milk and is therefore not expected to cause a nutritional imbalance in the diet of infants.

## F Information related to potential impact on consumer understanding and behaviour, including labelling changes

| Handbook Guideline | Requirement | Application section |
|---|---|---|
| 3.3.3 – Nutritive substances | G.1 Information to demonstrate the level of consumer awareness and understanding of the nutritive substance in the foods | Section F.1 |
| | G.2 Information on the actual or potential behaviour of consumers in response to proposed foods | Section F.2 |
| | G.3 Information to demonstrate that the consumption of foods containing the nutritive substance will not adversely affect any population groups | Section F.3 |
| 3.6.2 – Special purpose foods – Infant formula products | C.1 Information related to safety or nutritional impact of the proposed labelling change | Section C |
| | C.2 Information to demonstrate that the proposed labelling change will be understood and will assist consumers, if applicable | Section F.1 |

### F.1 Information to demonstrate the level of consumer awareness and understanding of the nutritive substance in the foods

Standard 2.9.1 of the Code includes labelling requirements for infant formula products. Information about the presence of an added nutritive substance can only be made on the label of an infant formula product in relation to a statement of ingredients and a declaration of nutrition information. Standard 1.2.7 – Nutrition, health and related claims prohibits nutrition content and health claims being made about infant formula products. The potential impact on labels of infant formula products containing added GOS/ITF and 2'-FL is therefore expected to be minimal and restricted to the ingredients in the ingredients list and listing the quantity of the ingredients in the declaration of nutrition information (along with other nutrients, including vitamins, minerals and any other substance used as a nutritive substance). Both ingredients are permitted to be added in isolation to infant formula products in Australia and New Zealand. For consumers who are aware of the composition of infant formula products, the only difference to existing infant formula products will be that both ingredients will be listed on a label. Consumers may be aware and understand the positive benefits that can result from addition of the ingredients in infant formula via the healthcare system or information available in the public domain.

### F.2 Information on the actual or potential behaviour of consumers in response to proposed foods

The availability of infant formula products containing a combination of 2'-FL and GOS/ITF is unlikely to change consumers use of infant formula products in general, particularly in relation to decisions about breastfeeding compared to the use of infant formula products. Consumers may choose infant formula products for their infants based on these ingredients being permitted to be added. Consumers (parents and carers of infants) may become more aware of these ingredients in infant formula and their benefits from discussion with their healthcare professional and therefore may elect to choose products that contain these ingredients.

### F.3 Information to demonstrate that the consumption of foods containing the nutritive substance will not adversely affect any population groups

Infant formula products are tightly regulated in Australia and New Zealand in relation to composition to ensure that formula-fed infants are provided with adequate nutrition. The Code's permissions to add 2'-FL and GOS and ITF to infant formula products in Australia and New Zealand are evidence that these ingredients are safe and will not adversely affect infants. This application demonstrates the addition of both 2'-FL and a mixture of GOS/ITF to infant formula products is also safe for infants and provides benefits for formula-fed infants that approaches the benefits that HMOs provide for breastfed infants. No adverse effects from consumption of infant formula products containing both 2'-FL and GOS/ITF are expected, based on the information provided in section C above.

## G. Information related to internationally recognised standards, codes of practice, recommendations and guidelines

- Official Journal of the European Union: Commission Implementing Decision (EU) 2016/375
- Official Journal of the European Union: Commission Implementing Decision (EU) 2016/376
- Official Journal of the European Union: Commission Implementing Decision (EU) 2017/2201). This includes the Chr. Hansen (former Jennewein) 2'-FL (per A1190). Regulation (EU) 2017/2470
- FAO/WHO Codex STAN 72-1981 Standard for Infant Formula and Formulas for Special Medical Purposes Intended for Infants. Revision 2007.
- FAO/WHO Codex CXS 156-1987 Standard for Follow-Up Formula, Revision 2017.
- Australia New Zealand Food Standards Code – Standard 2.9.1 – Infant formula products
- Australia New Zealand Food Standards Code – Standard 1.2.7 – Nutrition, health and related claims

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